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REVIEW

Extracellular Vesicles as Potential Biomarkers in Addictive Disorders

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Abstract—Small extracellular vesicles (sEVs) and their role in mental and addictive disorders are extremely promising research areas. Because of their small size, sEVs can pass through the blood–brain barrier. The membrane of sEVs contain proteins that protect them against destruction by the organism's immune system. Due to these properties, sEVs circulating in the blood can be used as potential biomarkers of processes occurring in the brain. Exposure to psychoactive substances *in vitro* and *in vivo* affects sEV biogenesis and significantly alters the amount of sEVs and chemical composition of their cargo. Based on the published reports, sEVs carry numerous potential biomarkers of addictive pathologies, although the diagnostic significance of these markers still has to be evaluated. A large body of evidence indicates that psychoactive substances influence Rab family GTPases, Toll-like receptors, complement system components, and cytokines. In some studies, the effect of psychoactive substances on sEVs was found to be sex-dependent. It has become commonly accepted that sEVs are involved in the regulation of neuroinflammation and interaction between glial cells and neurons, as well as between peripheral cells and cells of the central nervous system. Here, we formulated a hypothesis on the existence of two mechanisms/stages involved in the effect of psychoactive substances on sEVs: the "fast" mechanism that provides neuroplasticity, and the "slow" one, resulting from the impaired biogenesis of sEVs and formation of aberrant vesicles.

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INTRODUCTION

Addictive disorders are among the most pressing problem of modern society. According to the World Health Organization, the prevalence of alcohol use disorders is 14.8% among men and 3.5% among women in Europe and 11.5% among men and 5.1% among women in the United States. In Russia, 1.2 million people were under medical supervision for the alcohol dependence syndrome and alcohol abuse in 2020.

Abbreviations: DA, dopamine; DR, dopamine receptor; EV, extracellular vesicle; MVB, multivesicular body; ESCRT, endosomal sorting complex required for transport; PAS, psychoactive substance; sEV, small extracellular vesicle; TLR, toll-like receptors.

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Based on the information provided by the United Nations Organization, in 2021, there were ~296 million drug users and ~40 million people with substance use disorders worldwide [1]. In Russia, the number of patients under medical supervision for the addiction syndrome and drug abuse reached 341,000 in 2020 [2].

Although the terms “addiction” and “dependence” are not fully equivalent, the term “addictive disorders” has become more and more accepted in modern lexicon, substituting the more traditional term “dependence diseases”. Addiction, or dependence, is a chronic progredient disease that manifests itself as a pleasure-seeking behavior or cravings for a psychoactive substance (PAS), despite obvious negative consequences for an individual [3]. Addictive disorders are recognized as such based on the presence of common clinical symptoms manifested as specific features in the interaction between an individual and an object of addictive behavior [3, 4]. It is currently believed that a significant role in the pathophysiology of addictive disorders belong to alterations in signaling in the reward and stress response brain circuitries, in particular, in their architectonics, caused by morphological changes associated with the PAS-induced neuroplasticity [3].

Secretion of small extracellular vesicles (sEVs) is an important element of intercellular communication, beside the well-studied neurotransmitter systems [5]. sEVs are cell-derived vesicles of approximately hundreds of nanometers in diameter that are limited by the lipid bilayer and carry molecules of biological origin. Previously, it had been generally believed that sEVs are used by cells to get rid of unnecessary substances, but this point of view has now lost its popularity. The two well-studied types of sEVs – exosomes and ectosomes – are secreted by almost all body cells and can be detected in almost any biological fluid. Ectosomes and exosomes differ in biogenesis but have highly similar functional and structural properties. The classification of these vesicles is poorly developed [6]; for the sake of convenience, vesicles smaller than 200 nm are considered sEVs and those larger than 200 nm are considered large EVs [6].

An interest in sEVs is due to the following properties of these particles. The markers on the sEV membrane are recognized by macrophages, which allows sEVs to enter tissues and persist for a relatively long period of time without being destroyed by the immune system [7]. sEVs are able to penetrate through the histohematic barriers, including the blood–brain barrier [8]. Due to these two properties, sEVs are present in all biological fluids, from which they can be isolated for analysis. These features also determine an increasing interest in sEVs in the context of addictive and psychiatric disorders. In this review, we focused our attention on the studies of sEVs that might shed light on specific processes in the pathogenesis of addictive disorders and can potentially be used in solving various clinical challenges.

EXTRACELLULAR VESICLES

Biogenesis of extracellular vesicles (EVs). Biogenesis of sEVs has been described in detail in [9]. Exosomes are products of cell endolysosomal system. Primary endosomes mature to become, first, secondary endosomes and then multivesicular bodies (MVBs). After MVB fusion with the plasma membrane, intraluminal vesicles become exosomes [9]. In another pathway, MVBs fuse with lysosomes followed by the degradation of their content. The fate of MVBs largely depends on small GTPases of the Rab family located on the MVB membrane. The presence of Rab27 on the membrane results in the MVB fusion with the plasma membrane and secretion of exosomes [10], while the presence of Rab7 targets MVBs to the fusion with lysosomes and degradation of their cargo [11] (Fig. 1). However, depending on the conditions and type of cells, regulation of intracellular transport of MVBs may involve other members of the Rab family [12].

Ectosomes are formed when a section of the plasma membrane and some part of the cytoplasm directly bud off the cell [9]. Previously, ectosomes had been called microvesicles, or oncosomes. The process of ectosome secretion includes, first, formation of a microdomain containing lipids and proteins in the cell plasma membrane, and then budding of this section into the extracellular space [9]. There are several specific pathways of ectosome secretion, but to a large extent, the mechanisms of biogenesis and secretion of ectosomes and exosomes overlap [13].

Formation of sEVs involves diverse cellular machinery. The main specialized structure participating in the formation and transport of intracellular vesicles is ESCRT (endosomal sorting complex required for transport) [14] consisting of four components (ESCRT0-3), which sequentially bind to the sites on the plasma or endosomal membranes, recruit some specialized proteins, and finally, cause the newly formed vesicle to bud off [15]. In addition to this mechanism, vesicle formation involves the syndecan–syntenin–ALIX molecular cascade [16], tetraspanins [17], changes in the actin cytoskeleton [18], neutral sphingomyelinase [19], ceramide [20], and arrestin domain-containing protein (ARRDC1) [21]. Vesicle packaging involves several post-translational protein modifications, which are well-known due to their role in other intracellular processes. The best-studied of them is ubiquitination [22]. In other words, cells utilize many intracellular mechanisms for sEV biogenesis and secretion, instead of a particular specialized pathway. The mechanisms

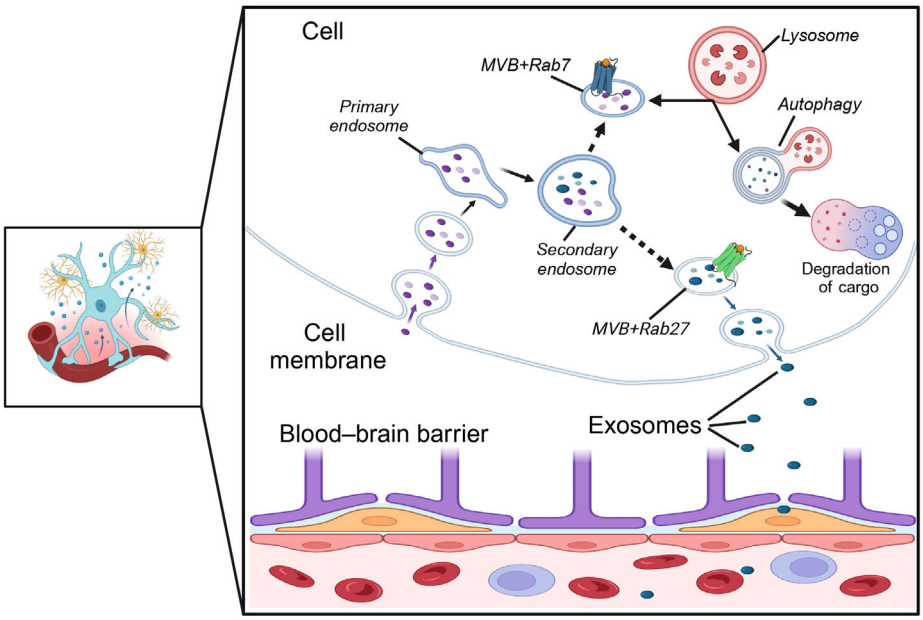

Fig. 1. Primary endosome matures and becomes secondary endosome and then an MVB. After the fusion of the Rab27-containing MVB with the plasma membrane, intralumenal vesicles become exosomes. The alternative pathway involves the fusion of the Rab7-containing MVBs with lysosomes and degradation of their cargo.

of formation and secretion of ectosomes and exosomes are largely the same. However, the heterogeneity of sEV population should be taken into consideration, since even a single cell can secrete sEVs by different mechanisms depending on its functional state.

The role of vesicles in cell-cell signaling. Recently, there has been a growing body of evidence that sEVs perform various important functions in a body. In particular, it was suggested that cells use sEVs to exchange information. This hypothesis has been confirmed in may studies, so that some researchers even speak about a special “vesicular signaling system” [23]. The idea that sEVs can act as mediators of cell–cell communication was formulated for the first time in the 1990s [24] based on the studies of information exchange between immune cells. It was found that antigen-presenting cells secrete major histocompatibility complex II proteins as component of exosomes. Although this process is rather slow, it allows antigen-presenting cells to present antigens to T cells.

Most examples of sEV-mediated communication have been obtained in brain cells. Moreover, from the structural point of view, it is easy to find evidence that brain sEVs form a neurotransmitter system, which, on one hand, is indisputably different from the classical neurotransmitter systems, but on the other hand, shares undeniable similarity with them [25]. sEVs secreted by oligodendrocytes stimulated axonal transport in the adjacent neurons [26]. Astrocyte-derived sEVs affected the spontaneous activity of neurons [27], significantly modified their induced activity [28], and altered the synaptic machinery morphology in these cells [29]. Neurons secrete sEVs that affect the functions of other neurons [30] and surrounding endothelial cells [31]. The studies of vesicle-mediated communication in the brain draw a similar picture: brain cells use sEVs to help neurons perform their functions; non-neuronal cells use sEVs to provide trophic support to neurons [32, 33]. However, in the case of long-term brain pathologies, sEVs can exert a deleterious effect on neurons [34].

The existence of sEV-mediated information exchange between body cells is currently beyond any doubt. However, the mechanisms of targeted delivery of sEVs, their internalization, and intracellular sorting have been studied rather poorly. So far, there are single works on the role of specific molecules in the recognition of sEVs by cells and on the role of particular component of sEVs in realization of their functions, which might be due to the fact that such experiments are technically difficult, and the capabilities of technologies used to study vesicles are often insufficient. However, these obstacles do not prevent the development of sEV-based diagnostic tests and even therapies [35].

Exosome- and ectosome-based diagnostics. Recent studies have opened new prospects for the practical use of exosomes and ectosomes. Diagnostics remains the main area requiring sEV application, although multiple attempts have been made to use these vesicles in therapy. Circulating sEVs can penetrate through the blood–brain barrier, while sEVs of the neuronal and glial origin are found in the peripheral blood. The latter allows to monitor changes

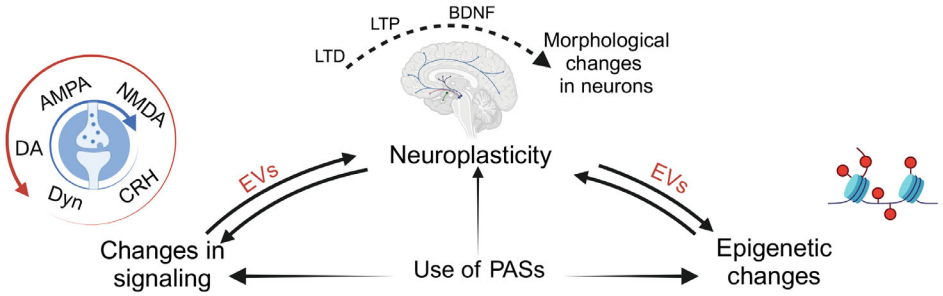

Fig. 2. The role of sEV signaling in the pathogenesis of PAS-induced disorders. BDNF, brain-derived neurotrophic factor; LTD, long-term depression; LTP, long-term potentiation; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxalopropionic acid; NMDA, N-methyl-D-aspartate; Dyn, dynorphin; CRH, corticotropin-releasing hormone; DA, dopamine.

occurring in the brain based on the composition of brain-derived EVs isolated from the peripheral blood.

The most impressive results for the clinical application of sEVs have been obtained in the diagnostics of cancer. This area has been studied quite extensively, and some of the findings are currently tested in clinical trials. The very first studies of sEVs in oncology resulted in the identification of surface proteins in sEVs derived from cancer cells [36], so these proteins have been investigated as potential biomarkers for cancer diagnostics. The functions of sEVs produced by cancer cell have also been studied. Thus, it was shown that sEVs derived from cancer cells suppressed immunity in premetastatic niches promoting further metastasis [37].

The therapeutic applications of sEVs are largely restricted to using stem cell-derived sEVs for the treatment of diseases [38]. Currently developed approaches are aimed at the isolation and culturing of stem cells with the purpose of collecting the maximum amount of sEVs [39]. The obtained vesicles can be preserved and used when necessary for therapy or pathology prevention.

sEVs IN ADDICTIVE DISORDERS

In psychiatry in general and in addiction studies in particular, the leading role in the pathogenesis of addition is attributed to the functional changes in the metabolism of neurotransmitters. It is believed that a generalized mechanism of neural response to PASs proceeds as follows [3]. PAS affects a cell and alters the functioning of one of the neurotransmitter systems. The activation of the cognate receptors induces a signaling cascade that triggers the synthesis of appropriate molecules. These molecules cause long-term changes in neurons, thus ensuring learning and memory processes, including those occurring in response to substance use (intoxication and tolerance) and anticipation of substance use (cravings). These changes are preserved and maintained at the molecular level due to the neuroplasticity associated with the substance use [40, 41]. Current studies confirm the fundamental role of glial cells in neuroplasticity. It is possible that sEVs serve as a link between the well-studied processes involved in the pathogenesis of addictive disorders (Fig. 2). sEVs act as transport vehicles for the efficient targeted delivery of required molecules because, due to the presence of specific surface proteins, they can reach the “addressees” directly, without affecting nontarget cells.

One of the functions of sEVs is involvement in the release of signaling molecules by astrocytes, resulting in long-term changes in neurons [28]. The second function is activation of microglial cells to provide pruning or other reactive changes [42]. The third function is sending messages to peripheral tissues [43].

At the same time, interactions between the neurons and glia are largely provided by ATP-dependent processes and depend on the ionic balance in the extracellular matrix [44]. It was found that astrocytes are involved in the formation of long-term memory in rats, as activation of gamma-aminobutyric acid (GABA) and acetylcholine receptors (and associated G proteins) in astrocytes affects the long-term memory consolidation through the long-term changes in the Ca^{2+} levels and modulation of the cFos expression [45]. It is known that activation of dopamine receptors (DRs) leads to the long-term changes in neural stem cells [46]. Moreover, the sensitivity to dopamine (DA) was found to be altered in neural stem cells of patients with psychiatric disorders compared to cells from mentally healthy individuals. Assis-de-Lemos et al. [46] have shown that the DA-induced changes involve mitochondrial hexokinase (mt-HK), an enzyme essential for cell energy metabolism. Therefore, DA may act as a regulator of energy metabolism in neurons, the sensitivity of energy metabolism to DA being dependent on genetic predisposition. Hence, impaired energy metabolism might be an important component in the pathophysiological mechanism of addictive disorders. Energy metabolism is also directly related to the sEV biogenesis, suggesting its association with changes in the sEV signaling in the pathology of addictive disorders.

In recent decades, the genetic basis of many psychiatric disorders has become the research focus of the scientific community. The most significant associations with addictive disorders have been demonstrated for the polymorphisms of genes coding for FTO (fat mass and obesity-associated protein), type 2 dopamine receptor (DRD2), and phosphodiesterase 4B (PDE4B) [47]. Gene expression is regulated by epigenetic mechanisms, such as post-translational modification of histones, DNA methylation, and expression of microRNAs (noncoding RNAs). Regulation of gene expression by noncoding RNA is closely related to sEVs, since these RNAs are among the most common cargo molecules carried by sEVs [48]. The role of sEVs in the dendritic spine density and number of DRs was confirmed in the studies of mice exposed to oxycodone *in utero* and during the postnatal period. It was found that an increase in the miR-504 content in brain sEVs led to a decrease in the dendritic spine density and upregulation of the DRD1 and DRD2 expression [49]. Predisposition to addiction to a great extent depends on the genetically determined features of CNS functioning. Thus, the genetic profile of 9 to 10-year-old substance-naive children with hereditary predisposition for addiction and mental disorders corresponded to a profile with a high polygenic risk of addiction [47]. It was found in animal models that epigenetic changes can persist in germ cells [50].

sEVs might be also important for the reproductive functions and preservation of epigenetic changes in the progeny. In particular, Lyu et al. [51] have shown that addition of exosomes from the semen of cocaine users and HIV-positive patients to cultured monocytes led to the cell adhesion, cytoskeleton reorganization, and chemotactic migration [51]. The role of sEVs in the semen has been poorly studied; they have been investigated mostly as putative markers of prostate cancer.

PASs affect many aspects of sEV biogenesis, as well as their size, number, and internalization. Thus, the number of fluorescently labeled neuronal exosomes internalized by microglial cells and astrocytes in the nucleus accumbens of mice self-administering cocaine was much smaller than in the controls [52]. The same team of authors had demonstrated that exosomes regulate the activity of the GLT1 glutamate transporter by transporting miR-124-3p from neurons to astrocytes [53]. The study conducted in mouse liver tissue, cultured cell, and human tissue samples has shown that exposure of hepatocytes to the increased levels of alcohol upregulated expression of miR-155, resulting in the suppression of autophagy and significant increase in the exosome production [54]. Incubation of microglial BV-2 cells with ethanol for 24 and 72 h decreased the number of exosomes depending on the duration of exposure, as well as increased the content of Rab7 and reduced the content of CD63 [55]. Self-administration of nicotine for 22 days by female rats increased the average size of sEVs (a similar effect was observed in male rats but did not reach the level of significance). Female rats also demonstrated an increase in the expression of genes involved in the ESCRT-dependent and independent pathways. In addition, there were multiple changes in the composition of protein cargo transported by sEVs [56]. Astrocyte-derived sEVs carrying miR-106b-5p [57] and sonic hedgehog (SHH) protein [58] affected ciliogenesis in astrocytes with a developed tolerance to morphine, indicating involvement of sEVs in the morphological changes of CNS cells.

The above data suggest that by impairing sEV biogenesis, PASs cause the appearance of aberrant sEVs that act on nontarget cells and organs or affect the target cells in an unwanted manner or with an unwanted strength. Immune system is one of the body systems negatively affected by PAS. In particular, the pathogenesis of PAS-induced changes includes, inter alia, direct effect of dopamine on the expression of inflammatory factors in immune cells, activity of immune cells, and even modulation of T cell differentiation [59]. Many studies have demonstrated that exosomes participate in cell–cell interactions as carriers of inflammatory mediators. Ibáñez et al. [60] found that changes in the content of exosomes isolated from cultured astrocytes exposed to ethanol for 24 h included an increase in the amounts of microRNAs (miR-146a) and inflammation-associated proteins (COX-2), as well as upregulated expression of inflammation-related genes (*IL-1B*, *Traf6*, *Mapk14*, *Foxo3*). However, the most important finding was that these changes were absent in the exosomes of astrocytes deficient by the Toll-like receptor 4 (TLR4) [60]. On the other hand, there are data that cocaine increases the content of miR-124, resulting in the reduced expression of TLR4 and other proinflammatory proteins [61].

In a large-scale study by Chand et al. [62], macaques and rats self-administering methamphetamine exhibited numerous changes in exosomes, including increase in their size from 100-200 nm to 50-500 nm, as well as upregulation of genes involved in the functioning of the ESCRT Alix complex. The authors also observed an increase in the content of exosomal surface proteins (Alix, TSG101, HSP70, and CD63), which they interpreted as changes in the ratio between particular subtypes of exosomes. The level of miR-29a also increased but only after a long-term exposure of cultured cells to methamphetamine and only in sEVs (but not in large EVs). The authors suggested that miR-29a acts through the activation of the TLR7 signaling and increase in the production of proinflammatory cytokines (IL-1β and TNFα) and CCR5 and CXCR3 receptors in the microglia [62]. Importantly, TLR4 and TLR7

not only participate in the inflammatory response, but are also involved in the activity of endosomal system, which relates their functions to the sEV biogenesis.

It was shown that in cultured cells, alpha-synuclein can be transported from neurons to astrocytes with the involvement of exosomes [63]. In monkeys self-administering oxycodone for three years, the content of neurofilament light chains in the exosomes derived from neurons and astroglia and isolated from the blood and the content of alpha-synulcein in the exosomes derived from neurons and isolated from blood were significantly increased [64]. This increase correlated with the reduced volume of frontal and parietal lobes. Rather surprisingly, the size of exosomes isolated from the blood of animals chronically exposed to oxycodone was 133 nm, i.e., significantly exceeded the size of exosomes in the blood of control animals (102 nm). The concentrations of several microRNAs in the exosomes derived from neurons, astroglia, and microglia differed in the control and chronically narcotized animals [64]. Sil et al. [65] observed an increase in the beta-amyloid level in the astrocytes of the frontal cortex and basal ganglia of morphine-dependent macaques. They reproduced this effect in cultured human astrocytes, confirming an increase in the number of beta-amyloid-carrying sEVs, as well as upregulation of expression of proinflammatory mediators [65]. Moreover, addition of exosomes derived from neurons, astroglia, and microglia to cultured monocytes induced a proinflammatory response. Because exosomes of chronically narcotized animals differed from the control vesicles in many parameters, the authors suggested that circulating brain-derived vesicles can be indicators of the severity of neurodegeneration in the brain, as well as some other processes. These parameters included microRNA levels, proinflammatory potential, and even vesicle size, i.e., characteristics seemingly unrelated to the biological effect of exosomes. In general, this study suggested that exosomes circulating in the blood can be sources of information about a wide variety of processes occurring in the brain during chronic narcotization. The relationship between sEVs detected in the peripheral blood and neuroinflammation has been confirmed experimentally. When exosomes derived from the plasma of mice injected with *Escherichia coli* lipopolysaccharide were intravenously administered into intact mice, the latter demonstrated upregulated expression of many inflammatory factors, as well as microgliosis and astrogliosis [66].

Caobi et al. [67] discovered dose-dependent changes in the content of inflammation-regulating microRNAs (miR-627-5p, miR-378e, miR-150-5p, miR-1290) in the exosomes produced by peripheral blood mononuclear cells isolated from healthy doors and then infected with HIV and exposed to morphine. For example, the level of miR-1290 was 12 times higher compared to the control [67]. Microglial exosomes isolated post mortem from the hypothalamus of rats exposed daily to alcohol were characterized by the increased levels of apoptotic factors, such as the complement protein C1q, membrane attack complex, and reactive oxygen species [68]. Cocaine impaired biogenesis and altered composition of exosomes, as well as affected expression of exosomal proteins by cultured microglial cell [69]. This psychostimulant also altered intracellular expression of small GTPases of the Rab family, presumably affecting intracellular vesicle transport. These alterations in the intracellular traffic, e.g., changes in the proportion of MVBs fusing with lysosomes for subsequent degradation, might have caused an observed decrease in the exosome secretion. The authors also revealed that cocaine decreased the viability of microglial cells. In another study, the content of ganglioside GD1a in brain-derived sEVs isolated from mice exposed to cocaine for 12 days increased in sEVs obtained from male (but not female) mice, suggesting the existence of sex differences in the effect of cocaine on the lipid composition of sEV [70].

Evaluation of microRNAs in human extracellular vesicles and experiments in mice have demonstrated that alcohol intoxication decreased the levels of anti-inflammatory microRNAs (miR-146a-5p, miR-21-5p, miR-182-5p) in plasma EVs from women and female mice, but increased their content in EVs isolated from males. In the cerebral cortex of female mice, ethanol downregulated the levels of miR-146a-5p and miR-21-5p, while simultaneously promoting expression of inflammatory target genes (*Traf6*, *Stat3*, and *Camk2a*) [71]. In a small study (6 patients with alcohol dependence and 6 healthy volunteers), 254 differentially expressed (149 upregulated and 105 downregulated) circular RNAs were identified in the plasma exosomes; one of them, hsa-circ-0004771, was suggested as a biomarker of alcohol dependence severity [72].

Chen et al. [73] examined 140 male patients that had been treated at the Kunming Medical University from January 2018 to October 2019: 60 patients with heroin addiction, 60 patients with methamphetamine addiction, and 20 healthy controls. The authors showed that the levels of exosomal hsa-miR-451a and hsa-miR-21a have a prognostic capacity (AUC, 0.966 and 0.861) for the heroin and methamphetamine use, respectively. An increased content of hsa-miR-744-5p was associated with the acute phase of withdrawal syndrome and positively correlated with the serotonin levels in patients with heroin and methamphetamine addiction [73]. The authors also found a relationship between miR-92a-3p, miR-363-3p, miR-16-5p, and miR-129-5p and the total score on the Hamilton Anxiety Rating Scale. The levels of miR-92a-3p, miR-363-3p, miR-16-5p, and miR-129-5p significantly correlated

Table 1. Potential biomarkers in sEVs

Source	Potential marker	Associated process	References
Rats administered with oxycodone for 14 days	miR-504	changes in the expression of dopamine receptors DRD1 and DRD2	[49]
Semen of HIV-positive drug users	reverse transcriptase, HIV p24 antigen, benzoylecgonine	HIV progression and cocaine use	[51]
Mice self-administering cocaine	impaired internalization of exosomes	impairment of neuronal–glial signaling	[52]
Mouse brain preparation	miR-124-3p	increased activity of astrocytic glutamate transporter	[53]
Cultured mouse and human liver cells exposed to ethanol	miR-155	reduced autophagy in hepatocytes and increased number of exosomes	[54]
Cultured microglial cells exposed to ethanol	increased concentration of Rab7; decreased concentration of CD63	impaired biogenesis of exosomes	[55]
Rat brain	males: cyclin Y, GABA-A γ2-long isoform, ATP-citrate synthase, dolichol-phosphate mannosyltransferase subunit 1, mesoderm-specific transcript homologue protein; females: aquaporin-1, ezrin, phosphatidylinositol 5-phosphate 4-kinase type 2α	various regulatory functions	[56]
Cultured astrocytes from mice with the developed tolerance to morphine	miR-106b-5p	activation of ciliogenesis through the expression of CEP97	[57]
Cultured astrocytes from mice with the developed tolerance to morphine	Sonic hedgehog (SHH) protein	increased number of astrocytes with a cilium; increase in the cilium length	[58]
Cultured astrocytes exposed to ethanol	COX-2; *IL-1B*, *Traf6*, *Mapk14*, and *Foxo3* genes; miR-146a	TLR4-mediated proinflammatory changes	[60]
Macaques and rats self-administering methamphetamine	Alix, TSG101, HSP70, and CD63; miR-29a; change in the size of exosomes	alteration in EV biogenesis, proinflammatory changes in microglia	[62]
SH-SY5Y cells exposed to methamphetamine	α-synuclein	cognitive deficit	[63]
Neuron- and astroglia-derived exosomes isolated from the blood of monkeys self-administering oxycodone for three years	α-synuclein, neurofilament light chains	cognitive deficit	[64]
Astrocytes obtained from the brain preparation of macaques administered with morphine	toxic forms of amyloid	cognitive deficit	[65]

Table 1 (*cont.*)

Source	Potential marker	Associated process	References
HIV-infected peripheral blood monocytes exposed to morphine	miR-627-5p, miR-378e, miR-150-5p, miR-1290	numerous putative signaling pathways associated with the modulation of inflammation	[67]
Cultured microglial cells exposed to ethanol	complement C1q protein	activation of proinflammatory pathways	[68]
Cultured microglial cells	expression of small GTPases of the Rab family	sEV biogenesis	[69]
Brain of mice administered with cocaine	increase in the GD1a ganglioside content in males	BDNF regulation	[70]
EVs from human and mouse females and males after exposure to ethanol	in female mice and women: downregulated expression of miR-146a-5p, miR-21-5p, miR-182-5p, miR-146a-5p, miR-21-5p; upregulated expression of *Traf6*, *Stat3*, and *Camk2a* genes	proinflammatory response	[71]
Patients with alcohol dependence syndrome	hsa-circ-0004771	modulation of various cellular processes	[72]
Patients with heroin and methamphetamine dependence syndrome; acute phase of heroin and methamphetamine withdrawal syndrome	differences between the addicts and control subjects in the contents of hsa-miR-451a and hsa-miR-21a and in the content of hsa-miR-744-5p during acute phase of withdrawal syndrome	modulation of various cellular processes	[73]
Plasma of patients with heroin and methamphetamine addiction	miR-16-5p, miR-129-5p, miR-363-3p, miR-92a-3p	synaptogenesis, cell adhesion, focal contacts, and major histocompatibility complex class II	[74]
Patients with methamphetamine withdrawal syndrome	miR-137	regulation of cell functions	[75]
Patients with heroin dependence during early (7-14 days) and prolonged (about a year) remission	during early remission: chemokines PF4 and PPBP; during prolonged remission: increased levels of mRNAs for CD74, HLA-E, SELENOH-205, RPL18-207, ABCA7, RIG-1-like receptor	immunity	[76]
Plasma of patients with cannabinoid dependence	properdin, SHANK1	regulation of inflammation and synaptogenesis	[77]
Plasma of HIV-positive alcohol and tobacco users	hemopexin, properdin	regulation of inflammation	[78]
sEVs isolated from the blood of HIV-positive alcohol and tobacco users	GFAP and L1CAM	proteins identifying the origin of sEVs	[79]

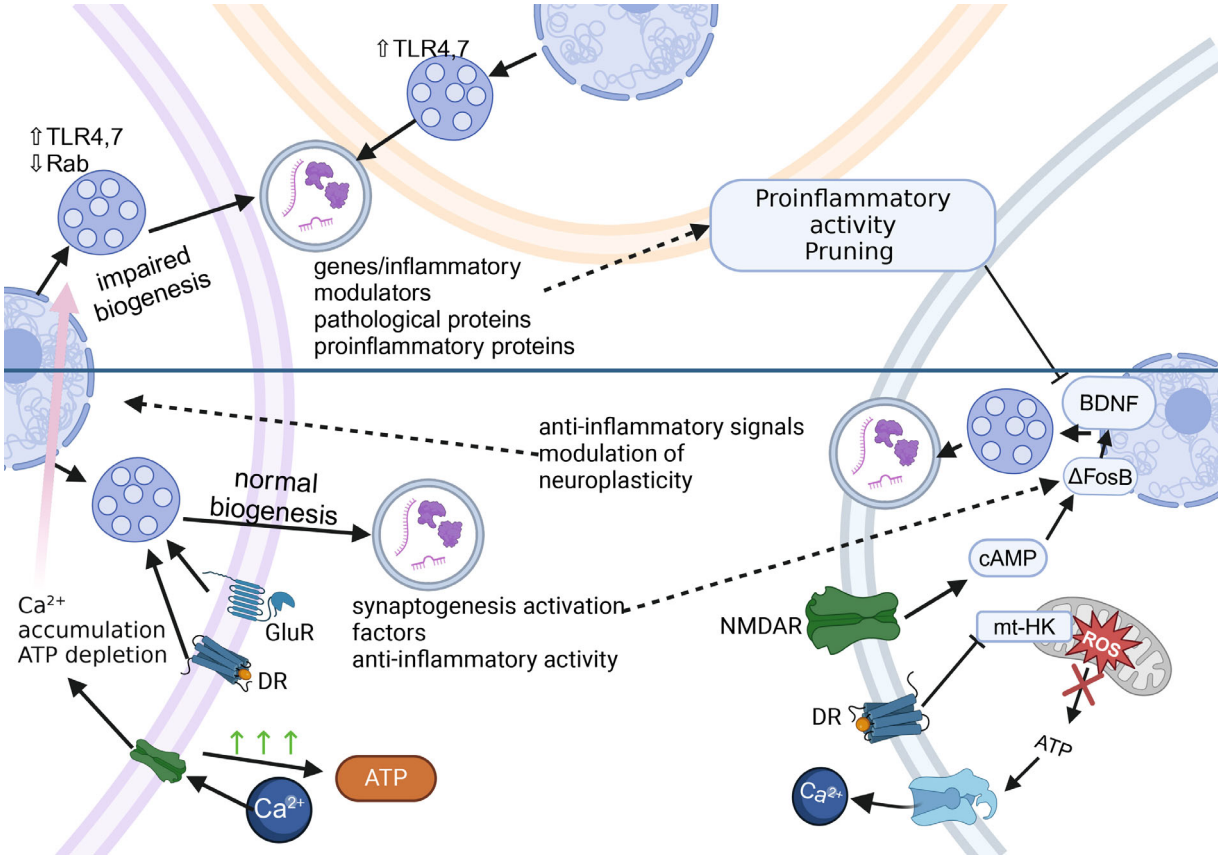

Fig. 3. Hypothesis on the sEV involvement in the pathogenesis of substance use disorders proposed by the authors of this review. The figure shows a system composed of astrocyte (on the left), microglia (at the top), and neuron (on the right). Below the dividing line, there are fast changes: (1) synaptogenesis induced by the first effects of PAS on the neuron triggers the release of sEVs containing cargo that reduces the activity of proinflammatory factors and "requests" for synaptogenesis support from the astrocyte; (2) in response to the "request" from the neuron, astrocyte releases sEVs containing anti-inflammatory factors and factors controlling synaptogenesis; (3) this provides consolidation of neural connections and reduction of microglial activity; (4) in parallel, PAS impairs cell metabolism, leading to the accumulation of calcium and reactive oxygen species in the cell. Above the dividing line, there are "slow changes": (i) dysregulation of energy metabolism and ionic imbalance lead to impaired vesicle biogenesis; (ii) as a result, production of proinflammatory factors and pathological proteins increases; (iii) this leads to the activation of microglia, reduced synaptogenesis, and development of neurophysiological impairments. ΔFosB, delta-FosB protein; BDNF, brain-derived neurotrophic factor; cAMP, cyclic adenosine monophosphate; GluR, glutamate receptor; NMDAR, N-methyl-D-aspartate receptor; mt-HK, mitochondrial hexokinase; ROS, reactive oxygen species.

with the total score on the Hamilton Depression Rating Scale in patients with methamphetamine addiction but not heroin addiction. Researchers believe that these microRNAs may have clinical application as diagnostic and prognostic biomarkers in various psychopathological states [74].

The study involving 37 methamphetamine-dependent patients and 35 healthy volunteers of comparable age and sex demonstrated a steady decrease in the concentration of miR-137 responsible for neurogenesis and maturation of neurons in sEVs from the peripheral blood of patients with methamphetamine withdrawal syndrome [75]. Investigation of the inflammatory response in 20 patients with heroin dependence abstaining for 7-14 days (early remission), 21 patients abstaining for approximately a year (prolonged remission), and 38 healthy volunteers demonstrated that early remission was characterized by the upregulated transcription of chemokines PF4 and PPBP. Prolonged remission was accompanied by an increase in the amount of RNAs coding for CD74, HLA-E, SELENOH-205, RPL18-207, ABCA7, and RIG-1-like receptor [76]. Exosomes in the blood of these patients contained long noncoding RNAs that can also be involved in the activation of proinflammatory response in patients with heroin dependence. In another study, brain-derived sEVs from patients with the cannabinoid dependence syndrome formed at the early age, contained increased amounts of properdin and SHANK1, proteins involved in the regulation of inflammation and synaptogenesis [77].

Kodidela et al. [78] have studied the groups of HIV-positive alcohol and tobacco users and found significant differences in the properdin concentration

in the sEVs from healthy volunteers and HIV-positive smokers and in the hemopexin concentration in sEVs from healthy subjects and HIV-positive alcohol users [78]. The same research team has shown that concentrations of GFAP and L1CAM in sEVs circulating in the peripheral blood of HIV-positive alcohol and tobacco users were higher compared to those in healthy volunteers [79]. They also revealed differences between the levels of interleukins and chemokines in the plasma and exosomes of HIV-positive smokers and drinkers and HIV patients who abstained from alcohol and tobacco. However, this study was carried out in a small sample (15 subjects only), which significantly complicates interpretation of the obtained results. Moreover, some of the studied cytokines have not been detected at all [80]. Table 1 summarizes all potential biomarkers described above.

The therapeutic potential of exosomes in substance use disorders has been demonstrated in a number of studies. For example, exosomes isolated from mesenchymal stem cells reduced alcohol intake, signs of oxidative stress, neuroinflammation, activation of astrocytes, and density of microglia and promoted GLT1 expression in the nucleus accumbens in rats [81]. Mellado et al. [82] have shown that intravenous administration of exosomes isolated from mesenchymal stem cells prevented neuroinflammation development in young mice by decreasing the activity of related genes, as well as reduced myelin and synapse damage, which had a positive effect on cognitive functions [82]. Intranasal administration of sEVs loaded with lincRNA-Cox2-siRNA suppressed proliferation of microglia induced by bacterial lipopolysaccharide [42].

Therefore, the effects of PASs on the sEV-mediated signaling are extremely versatile and involve signaling microRNAs present in sEVs and associated with the regulation of inflammation, alterations in the biogenesis of exosomes by microglial cells responsible for immune response, and increase in the content of pathological proteins. Unfortunately, most of the obtained data cannot be fully extrapolated onto clinical practice and leave a lot of room for speculation.

Based on the available data, we have formulated a hypothesis on the existence of two stages of sEV-mediated cellular response to PASs (Fig. 3). The first “fast” stage involves the binding of PAS to the cell receptors in the CNS and triggering of molecular cascades, eventually leading to changes in the biogenesis and loading of sEVs. These changes might underlie brain neuroplasticity, i.e., learning and memory.

The second “slow” stage might be associated with the accumulation of inflammation-like changes and impaired cell metabolism. At this stage, sEVs play the role of major modulators of inflammation, immune response activation, and, probably, sensitization of immune cells to their own antigens. These changes might be involved in the formation of cognitive deficit and impaired impulse control associated with the long-term use of PASs.

CONCLUSIONS

Based on the accumulated data, the studies of exosomes and other small extracellular vesicles in addictive disorders are a highly promising research area. The key advantage of vesicles freely circulating in the peripheral blood over other biological markers is their ability to penetrate through the blood–brain barrier. It will be reasonable to assume that detailed investigation of the role of sEVs in pathophysiological processes underlying addictive disorders will result in the discovery of multifunctional biomarkers. First, identification of specific differences between sEVs in healthy people and individuals with a history of addiction can ensure early diagnostics and detection of risk groups. Second, elucidation of changes in the processes involving sEVs against the background of established addiction can lead to the detection of targets for developing new therapeutic techniques. Third, monitoring sEVs might make allow to study the response to therapy not only based on clinical signs but also at the molecular level.

Contributions. V.V.S., A.A.Y., and M.A.V. developed concept and analyzed the available literature; V.V.S. and A.A.Y. wrote the manuscript; A.A.Y., V.V.S., and M.A.P. prepared figures; N.N.I. and M.A.V. edited the manuscript.

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