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Gliocrine System: Astroglia as Secretory Cells of the CNS

Nina Vardjan,

Laboratory of Neuroendocrinology-Molecular Cell Physiology, Faculty of Medicine, Institute of Pathophysiology, University of Ljubljana, 1000 Ljubljana, Slovenia

Vladimir Parpura,

Department of Neurobiology, The University of Alabama at Birmingham, Birmingham, AL, USA

Alexei Verkhratsky,

Faculty of Biology, Medicine and Health, The University of Manchester, Manchester M13 9PT, UK

Center for Basic and Translational Neuroscience, Faculty of Health and Medical Sciences, University of Copenhagen, 2200 Copenhagen, Denmark

Achucarro Center for Neuroscience, IKERBASQUE, Basque Foundation for Science, 48011 Bilbao, Spain

Robert Zorec

Laboratory of Neuroendocrinology-Molecular Cell Physiology, Faculty of Medicine, Institute of Pathophysiology, University of Ljubljana, 1000 Ljubljana, Slovenia

Celica Biomedical, 1000 Ljubljana, Slovenia

Abstract

Astrocytes are secretory cells, actively participating in cell-to-cell communication in the central nervous system (CNS). They sense signaling molecules in the extracellular space, around the nearby synapses and also those released at much farther locations in the CNS, by their cell surface receptors, get excited to then release their own signaling molecules. This contributes to the brain information processing, based on diffusion within the extracellular space around the synapses and on convection when locales relatively far away from the release sites are involved. These functions resemble secretion from endocrine cells, therefore astrocytes were termed to be a part of the gliocrine system in 2015. An important mechanism, by which astrocytes release signaling molecules is the merger of the vesicle membrane with the plasmalemma, i.e., exocytosis. Signaling molecules stored in astroglial secretory vesicles can be discharged into the extracellular space after the vesicle membrane fuses with the plasma membrane. This leads to a fusion pore formation, a channel that must widen to allow the exit of the Vesiclar cargo. Upon complete vesicle membrane fusion, this process also integrates other proteins, such as receptors, transporters and channels into the plasma membrane, determining astroglial surface signaling landscape. Vesiclar cargo, together with the whole vesicle can also exit astrocytes by the fusion of multivesicular bodies with the plasma membrane (exosomes) or by budding of vesicles (ectosomes) from the plasma membrane into the extracellular space. These astroglia-derived extracellular vesicles can later interact with various target cells. Here, the characteristics of four types of astroglial secretory vesicles: synaptic-

like microvesicles, dense-core vesicles, secretory lysosomes, and extracellular vesicles, are discussed. Then machinery for vesicle-based exocytosis, second messenger regulation and the kinetics of exocytotic vesicle content discharge or release of extracellular vesicles are considered. In comparison to rapidly responsive, electrically excitable neurons, the receptor-mediated cytosolic excitability-mediated astroglial exocytotic vesicle-based transmitter release is a relatively slow process.

Keywords

Exocytosis; Astrocytes; Fusion pore; Gliocrine system; Secretory vesicles

4.1 Vesicular Network and Astroglial Secretion

Similarly to all eukaryotic cells, astrocytes (homoeostatic glial cells of the central nervous system, CNS), contain a complex cytoplasmic network of vesicles. Lysosome, a vesicular organelle discovered in 1955 [29], is present in astrocytes, and plays a prominent intermediate role in endo- and exocytotic vesicle pathways (Fig. 4.1) [152]. It has been hypothesized almost a century ago, that astrocytes act as secretory cells, when in 1910 Jean Nageotte, based on the microscopic observations, considered that astrocytes act as secretory cells [90]. In the last two decades, using a variety of experimental approaches (e.g., by optical and membrane capacitance measurements, electrochemical amperometry, and selective interference with proteins of the exocytotic machinery), it has been determined that astrocytes can release signaling molecules via a vesicle-based mechanism (i.e., exocytosis) and are thus actively involved in information processing in the brain [136, 139]. Although being electrically non-excitable, astrocytes, similarly to neurons, possess (i) exocytotic vesicles, (ii) express proteins for regulated SNARE (Soluble NSF Attachment protein REceptor)-dependent vesicular exocytosis and (iii) can respond to various extracellular stimuli with an increase in cytosolic second messengers triggering Vesiclar exocytosis. The SNARE components of exocytotic machinery in astrocytes are not identical to neurons, nor are the vesicle types, their fusion sites and regulation of exocytosis [77, 86, 136, 139, 152, 153].

4.2 Astroglial Secretory Vesicles

Astrocytes contain various different types of secretory vesicles loaded with different types of molecules (such as ATP, D-serine, glutamate, atrial natriuretic peptide (ANP), brain-derived neurotrophic factor (BDNF), etc., Fig. 4.2) [39, 40, 101, 139]. These secretory vesicles are classified into synaptic-like microvesicles (SLMV), [8, 12, 27, 58], dense-core vesicles (DCVs) [17], secretory lysosomes (SL) [71], and extracellular vesicles (EVs) [38].

4.2.1 Synaptic-Like Microvesicles

Astroglial SLMVs are clear electron-lucent vesicles, which are similar to neuronal synaptic vesicles [28, 60], their diameters range between 30 and 100 nm and these SLMVs store low molecular weight signaling molecule glutamate (~147 Da) and in some astrocytes also D-serine (~105 Da). In hippocampal slices, larger SLMVs have been identified in astrocytes

(1–3 μm in diameter), which may be generated by intracellular fusion of smaller vesicles or other organelles or both upon sustained Ca^{2+} or mechanical stimulation [59], perhaps a manifestation of a pathological status. D-serine has been recently proposed to reside preferentially in neurons, since biosynthetic enzyme of D-serine serine racemase is expressed almost entirely by neurons [97, 145], with astrocytes arguably being the source of L-serine, which cannot be synthesized in neurons. As revealed with electron microscopy, astrocytes with SLMVs lack the structurally organized active zones with clearly defined synaptic vesicle pools with hundreds to thousands of synaptic vesicles (SVs) per synapse (the readily releasable and the reserve vesicle pools), which are typically found in presynaptic neurons [12, 58]. However, SLMVs in astrocytes do organize in small spaced clusters (2–15 vesicles) located near the astrocytic plasma membrane of the perisynaptic astrocytic process. Endoplasmic reticulum appears located in close proximity to these clusters, suggesting that astrocytes contain functional nanodomains, where a local Ca^{2+} increase can trigger release of glutamate and/or D-serine [9, 12, 58, 82]. However, astrocytic perisynaptic processes are mainly devoid of subcellular organelles [105, 124].

Whether signaling molecules glutamate and D-serine are stored inside the same astroglial SLMVs is still a matter of debate. In cultured astrocytes vesicular SNARE protein vesicle-associated membrane protein 2 (VAMP2) and cellubrevin (VAMP3) were found colocalized with both vesicular glutamate transporters (VGluTs; [12, 14, 87]) and D-serine [80, 88], while studies on tissue astrocytes showed that glutamate and D-serine can be stored in distinct SLMVs within the same astrocyte [9]. Examination of immunopurified astroglial SLMVs showed that SLMVs can co-store both glutamate and D-serine [82]. The observation that isolated astroglial SLMVs and isolated neuronal SVs contain different signaling molecules (isolated SLMVs contain D-serine and glutamate [27, 82] and isolated neuronal SVs contain glutamate, glycine GABA and are devoid of D-serine [82, 125]) (although D-serine has been recently proposed to reside preferentially in neurons [97]) might indicate distinct physiological roles of SLMVs and SVs in the CNS.

SLMVs use VGluTs to move glutamate from the cell cytosol into vesicular lumen using a H^{+} gradient, created by vacuolar-type H^{+} -ATPase (V-ATPase), with associated chloride flux. VGluTs 1, 2, and 3 were identified in the membrane of SLMVs in astrocytes in culture, and VGluTs 1 and 2 were shown to associate with SLMVs in tissue astrocytes of several brain areas of hippocampus (CA1), cerebral cortex, striatum, dentate-molecular layers [12, 82, 87, 92, 148], although VGluTs 1–3 were not identified in tissue astrocytes from mice grey matter, thalamic ventrobasal nucleus primary somatosensory cortex, hippocampus and cerebellum [70], suggesting that astrocytes from different brain regions may carry different vesicle types consistent with the regional and functional heterogeneity of astrocytes [91]. Vesicular D-serine transporters (VSerT) were identified in immunopurified astrocytic vesicles. They are likely D-serine/chloride co-transporters and use the H^{+} gradient created by V-ATPase to refill the vesicles with D-serine [81, 82].

4.2.2 Dense-Core Vesicles

Astroglial dense-core vesicles (DCVs) are ultrastructurally similar to the large-dense core vesicles (LDCVs) that release neuropeptides and hormones from neuroendocrine cells [16]

and neurons [60]. Although DCVs are not very abundant in astrocytes [27], both DCVs and SLMVs can coexist within the same astrocyte [94, 113]. Moreover, DCVs appear larger (100–600 nm; [17, 54, 109]) than SLMVs. The DCVs in cultured astrocytes may contain secretogranins II [17, 94, 109] and III [95], chromogranins [54], ANP [61, 94], neuropeptide Y [109, 113], and ATP [24, 96]. Secretogranins containing DCVs were identified also in astrocytes in human brain tissue [54], indicating the presence of DCVs in astrocytes in situ. Inositol-1,4,5-triphosphate (IP₃) receptors (IP₃Rs), acting as IP₃-gated Ca²⁺ channels were detected on DCV membranes in astrocytes in brain tissue suggesting that DCVs also serve as IP₃-sensitive intracellular Ca²⁺ stores [54].

Using a super-resolution microscopy approach it was shown that the peptidergic ANP- and BDNF-containing vesicles have diameters less than 100 nm [39] and that the ANP-antibody retrieving vesicles do not exhibit a dense core [107]. Also, astrocytes contain fewer smaller and less dense secretory granules containing secretogranin II [17]. Thus, it appears that peptidergic granules in astrocytes are not uniform in morphological appearance.

Tissue-type plasminogen activator (tPA) is considered to be released by neurons but taken up by astrocytes, possibly into recycling vesicles as these vesicles can uptake ANP-antibodies [107]. Interestingly, tPA is constitutively endocytosed by astrocytes via the low-density lipoprotein-related protein receptor, and is then exocytosed in a regulated manner. Extracellular glutamate inhibits the exocytotic recycling of tPA by astrocytes and on the other hand, capturing extracellular tPA into astrocytes reduces the NMDA-mediated responses potentiated by tPA [20].

4.2.3 Secretory Lysosomes

Secretory lysosomes with diameters between 300 and 500 nm [23, 150] that store signaling molecule ATP, have been identified in cultured astrocytes [56, 71, 96, 150]. Secretory lysosomes in astrocytes as in other cell types are likely involved in membrane repair [3]. Astroglial secretory lysosomes express lysosomal-specific markers, including cathepsin D and lysosomal-associated membrane protein 1 (LAMP1 [150]), monomeric GTP-protein Rab 7, SNARE protein tetanus neurotoxin (TeNT)-insensitive VAMP (TI-VAMP/VAMP7), which contributes to TeNT-independent exocytotic release of ATP [138], and vesicular nucleotide transporter VNUt [120], which is involved in ATP storage [93] within secretory lysosomes in astrocytes and hence warranting ATP release [63] from these astrocytic secretory organelles. Secretory lysosomes in astrocytes can be specifically labeled with dextrans [55, 134], FM dyes, and by a fluorescent ATP analogue MANT-ATP [150]. They may coexist with SLMVs in the same astrocyte [72]. Fusion of secretory lysosomes is regulated and induced with slow, locally restricted Ca²⁺ elevations [71], which are distinct from Ca²⁺ spikes inducing SLMV fusion [138].

4.2.4 Extracellular Vesicles: Exosomes and Ectosomes

Exosomes and ectosomes are extracellular vesicles (EVs) released from cells to deliver signals to target cells (Fig. 4.3). EVs control different biological processes by transferring membrane proteins, lipids, signaling molecules, mRNAs, microRNAs (miRNAs), and activating receptors of recipient cells, possibly playing a role in autocrine regulation.

Exosomes are released by exocytosis of multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs) that are called exosomes when released from cells into the extracellular space. Ectosomes (also called microvesicles) are assembled by outward budding of the plasma membrane and released (shed) from the plasma membrane into the extracellular space. Exosomes are vesicles of 50–100 nm in diameter, while ectosomes are larger vesicles from 100 to >1,000 nm in diameter [25]. Astrocytes release both types of EVs [38].

Ectosomes carrying interleukin-1 β (IL-1 β) may shed from cultured astrocyte upon ATP stimulation through activation of ionotropic purinoreceptor P2X₇. This is associated with rapid activation of acid sphingomyelinase, which moves from luminal lysosomal compartment to the plasma membrane outer leaflet altering membrane structure/fluidity leading to vesicle blebbing and shedding 1–2 min after ATP stimulation [13]. Diameters of ectosomes shed by cultured astrocytes vary between 100 and 1,000 nm [13, 110]. Moreover, upon repetitive ATP stimulation cultured astrocytes release vesicles from the cell surface that can be from 1 up to 8 μ m in diameter and express on their surface β 1-integrin proteins and contain mitochondria and lipid droplets together with ATP [34]. Although it has not been directly demonstrated [34], these vesicles likely represent ectosomes due to their large size. In addition to interleukin-1 β (IL-1 β) [13], mitochondria, lipid droplets, and ATP [34], culture astrocyte-derived ectosomes may also carry fibroblast growth factor 2 and vascular endothelial growth factor [110], ectoenzyme nucleoside triphosphate diphosphohydrolases that hydrolyze extracellular nucleotides [21], and matrix metalloproteinases and their inhibitors [121]. Ectosomes shed from astrocytes in response to lipopolysaccharide-induced stress contain miRNA miR-34a that enhances the vulnerability of dopaminergic neurons to neurotoxins by downregulating the anti-apoptotic protein Bcl2 [78]. Recently, it has been shown that cytokines tumor necrosis factor α and IL-1 β can modify the miRNA cargo of EVs shed from astrocytes to regulate neurotrophic signaling in neurons [22]. Astrocyte also shed EVs that promote transmigration of leukocytes into the brain through regulation of the peripheral acute cytokine response to IL-1 β -induced inflammatory brain lesion [31].

Exosomes containing heat-shock protein 70 are released from cultured astrocytes in response to oxidative and heat stress, suggesting a mechanism by which astrocytes provide antioxidant protection to neurones [132]. Retinal astrocytes release exosomes that contain anti-angiogenic components that inhibit laser-induced choroidal neovascularization [43]. Exosomes secreted from astrocytes carrying synapsin I promote neurite outgrowth and neuronal survival [142]. Astrocyte-derived exosomes have been reported to contain mitochondrial DNA [41] and may carry also disease-specific cargo and promote neurological disorders by spreading pathology. Indeed, cultured astrocytes expressing mutant copper-zinc superoxide dismutase 1 (SOD1) secrete exosomes, which carry mutant SOD1. Astroglial derived mutant SOD1-positive exosomes can transfer mutant SOD1 to cultured neurons and induce motor neuron death. This suggests a role of EVs in the pathogenesis of amyotrophic lateral sclerosis [6]. Moreover, cultured astrocytes exposed to amyloid peptide release exosomes enriched with pro-apoptotic ceramide and prostate apoptosis response 4 (PAR4). These exosomes are taken up by astrocytes and promote their apoptosis suggesting that exosome-mediated astrocyte death may contribute to neurodegeneration in Alzheimer's disease. Exosome-mediated miRNA transfer from

astrocytes to neurones has been suggested to participate in HIV-associated neurological disorders. Treatment of cultured astrocytes with pathogenic HIV trans-activator of transcription (Tat) protein and morphine triggers shuttling of miRNA miR29b via exosomes to neuronal cells, which results in decreased trophic factor platelet-derived growth factor (PDGF)-B expression and neuronal viability [52]. Nef (Negative Regulatory Factor), a protein encoded by primate lentiviruses such as HIV-1, has been shown to be released in EVs derived from astrocytes and human microglia and may accumulate in neighboring cells (Fig. 4.3) contributing to Nef-mediated neurotoxicity [118, 129]. Interestingly, this release appeared inhibited by elevated cytosolic calcium in human microglia [129]. Recently, it has been shown that reactive astrocytes release vimentin, an intermediate filament of the cytoskeleton, via exosomes. This promotes binding of exoenzyme *Clostridium botulinum* C3 transferase (that enzymatically inhibits small GTPases of the Rho family) to neuronal surface, which can be then internalized and promotes neuronal plasticity and growth [1].

As the field of studying EVs is still developing, it is important to note that when studying the internalization of EVs into cells, the methods and approaches have to be evaluated carefully. For example, when monitoring the internalization of EVs into astrocytes, EVs were labeled by a fluorescent dye PKH26, and it has been reported that a significant false-positive signal due to internalization of PKH26-nanoparticles was observed (Fig. 4.3), which can compromise the interpretation of EV internalization [112]. Thus, for EV uptake and functional studies it is critical to consider potential artifacts, since EVs are very small, often below the optical microscopy resolution.

4.3 SNARE and SNARE-Associated Proteins in Astrocytes

Astrocytes express vesicular R-SNARE and plasma membrane Q-SNARE proteins (Fig. 4.4). R-SNARE proteins synaptobrevin 2 (VAMP2), VAMP3 [27, 74, 80, 88, 99, 144], and TI-VAMP/VAMP7 [138] and Q-SNARE proteins SNAP23 and syntaxins 1, 2, 3, and 4 [48, 94, 148] have been identified in astrocytes as well as SNARE-associated proteins Munc18 [94] and synaptotagmin 4 [147]. In mammals, synaptotagmin 4 is not a Ca^{2+} -sensor for regulated exocytosis like synaptotagmin 1 in neurones is [143], but is important in modulating Ca^{2+} -evoked exocytosis [131]. SNARE proteins VAMP2 [144], VAMP3 [8, 12, 58, 123, 148], TI-VAMP/VAMP7 [138], SNAP23 [123], and syntaxin 1 [123] were confirmed also in brain tissue astrocytes using immunogold cytochemistry and confocal microscopy. Expression of synaptotagmins and other SNARE-associated proteins, such as Sec1/Munc18-like proteins, in brain tissue astrocytes still needs to be determined, although studies examining mRNA of astrocytes in brain tissue suggest expression of several synaptotagmin isoforms [84, 149] and SNARE-associated proteins [149].

The ternary SNARE fusion complex between vesicular and plasma membrane SNAREs [35, 36] in astrocytes is likely made of vesicular SNARE proteins VAMP2/3 (SLMV)s or TI-VAMP/VAMP7 (secretory lysosomes) and the plasma membrane SNAP23 and syntaxins [45, 85]. The formation of up to five SNARE complexes containing VAMP2 is believed to be sufficient to carry a single vesicle fusion in astrocytes [127].

Astroglial VAMP2 and VAMP3 colocalize with ATP [74] or D-serine [80]-storing vesicles. VAMP3 in astrocytes colocalizes also with the VGLUT1 and 2, vesicular glutamate

transporters present on SLMVs that store glutamate [8, 12, 58, 148], and likely D-serine [82]. TI-VAMP/VAMP7 is present in the membrane of the astroglial late endocytic/lysosomal compartments [138] storing ATP [4, 24, 96, 150].

The functionality and physiological role of exocytotic apparatus in astrocytes consisting of aforementioned SNARE proteins has been addressed and confirmed in multiple studies. It has been shown that cleavage of SNARE proteins with tetanus (TeNT) and botulinum neurotoxins (BoNT) in cultured astrocytes attenuates exocytotic release of glutamate [4, 10, 11, 12, 53, 87, 104] as well as a reduction in membrane capacitance (C_m) increases [39, 61] and in amperometric spikes [23], implying the role of SNARE proteins in the release of glutamate from cultured astrocytes. The inactivation of VAMP2/VAMP3 in astrocytes by TeNT abolishes the release of glutamate or D-serine from astrocytes in brain tissue slices [47, 58, 106]. Additionally, in a mouse model in which a dominant negative SNARE transgene is expressed in astrocytes to interfere specifically with astroglial VAMP2/3 [44, 103] the synaptic transmission and plasticity in these animals were altered [49, 63, 89, 103, 133]. Furthermore, in mice with targeted expression of BoNT/B in Müller cells, a subtype of astroglia that expresses BoNT/B-sensitive VAMP2/3, the disruption of the Ca^{2+} -dependent vesicular glutamate release from Müller cells was observed [128]. Moreover, it has been shown in mice with inducible expression of TeNT in astrocytes that TeNT-sensitive vesicular release from astrocytes is necessary for sustaining gamma oscillations associated with recognition memory in mice [68]. Interestingly, the use of various botulinum toxins and dominant negative SNARE peptides has demonstrated that SNARE proteins determine the fusion frequency of individual vesicles monitored by the high-resolution membrane capacitance technique [39]. All these data clearly imply that SNARE-mediated exocytosis is present in astrocytes and essential for normal brain function.

4.4 Regulation and Kinetics of Secretory Vesicle Release in Astrocytes

4.4.1 GPCR-Mediated Regulation of Secretory Vesicle Release: Ca^{2+} and CAMP Signals

Neurons are electrically excitable and release neurotransmitters from synaptic vesicles in synaptic terminals in response to depolarization. In contrast to neurons, astrocytes are electrically silent and display only receptor-mediated cytosolic excitability. Astrocytes sense extracellular signalling molecules via plasma membrane receptors. They express a large number of various types of receptors and many of these receptors are metabotropic high affinity G-protein-coupled receptors (GPCRs) [2, 100, 140, 151]. Binding of signalling molecules to these receptors may increase cytosolic levels of free Ca^{2+} as well as other astrocytic cytosolic secondary messengers, including the cyclic adenosine monophosphate (cAMP). Such cytosolic excitability may lead to secretory vesicle release of signaling molecules from astrocytes (see Vesicular Network). These gliosignalling molecules can then interact with the receptors on neurons affecting neuronal excitability [17, 100] or affect receptors on other neighbouring cells.

Stimulation of astroglial GPCRs coupled to G_q protein leads to increases in intracellular levels of cytosolic Ca^{2+} . Activation of G_q GPCRs triggers IP^3 signaling cascade that releases Ca^{2+} into the cytosol from the IP^3 -sensitive intracellular organelles acting as Ca^{2+} stores, such as endoplasmic reticulum (ER) [53, 69] and secretory vesicles [54]. Mitochondria can

modulate those cytosolic calcium dynamics in astrocytes by taking up Ca^{2+} from the cytosol or releasing this ion into the cytosol at time of high or low Ca^{2+} cytosolic levels, respectively [115, 126]. Ca^{2+} can also partially enter astrocytes from the extracellular space through voltage-gated Ca^{2+} channels [67, 73, 102], ionotropic receptors [64], sodium-calcium exchanger [116] and through the transient receptor potential canonical type 1-containing channel [75]. G_q -induced cytosolic Ca^{2+} increases in astrocytes occur as oscillations or sustained elevations [100, 141, 151], spontaneously or in response to signaling molecules [26]. Astrocytes can intercellularly communicate through gap junction channels. They can propagate cytosolic Ca^{2+} excitability by diffusion of IP_3 or Ca^{2+} through gap junctions to neighboring unstimulated astrocytes in the form of intercellular Ca^{2+} waves [122]. They can also release glutamate or ATP in response to Ca^{2+} excitability [15, 26, 42].

Stimulation of astroglial GPCRs coupled to G_s proteins activates adenylyl cyclase (AC), an enzyme catalyzing the conversion of ATP to cAMP [114, 135]. cAMP activates a number of effectors in the cell, primarily cAMP-dependent protein kinase A, but signalling via cAMP-activated GTP-exchange protein [30], cAMP-gated ion channels, and Popeye domain-containing proteins [37] may also be triggered [7]. G_s protein activation induces persistent cAMP elevations [135, 137], which are at least in the case of adrenergic receptor activation 10-fold slower compared to G_q protein-triggered Ca^{2+} elevations [50, 51]. Whether G_s -induced cAMP excitability can be propagated via gap junctions needs to be evaluated [33]. It has been suggested that G_q - and G_s -mediated pathways in astrocytes can interact, since G_s -signaling pathway may enhance G_q -mediated Ca^{2+} responses and vice versa [5, 50, 51, 57].

GPCR G_q - and G_s -protein signalling pathways were shown to be involved in the regulation of secretory vesicle release of chemical messengers from astrocytes. Ca^{2+} elevations in astrocytes trigger the release of glutamate [10, 12, 98, 104, 148], ATP [4, 24], secretogranin II [17], ANP [62], and D-serine [88] from secretory vesicles. cAMP elevations can trigger the release of secretogranin II from astroglial peptidergic vesicles [17]. In astrocytes pretreated with the membrane-permeable cAMP analogue dibutyryl-cAMP the Ca^{2+} -triggered release of ANP from secretory vesicles was enhanced [94]. cAMP might trigger the fusion of secretory vesicles de novo or it may modulate the fusion pore dynamics of already pre-fused secretory vesicles by increasing the diameter and open time of a fusion pore between the vesicle and plasma membranes, which needs to be still determined. The latter mechanism has been observed in neuroendocrine cells [18].

4.4.2 Kinetics of Secretory Vesicle Content Release in Astrocytes

Temporal dynamics of secretory vesicle release from cultured astrocytes has been monitored using (i) electrophysiological techniques (amperometry [23] and membrane capacitance (C_m) measurements [61, 117] in combination with UV-flash photolysis-induced increases in cytosolic Ca^{2+} levels [61]), and (ii) optical techniques (real-time confocal microscopy and total internal reflector fluorescence microscopy, TIRFM) in combination with fluorescent markers of vesicular cycling/fusion, such as FM dyes [71, 72, 150], acridine orange [12, 32], quinacrine [96, 111], fluorescent dextrans [56], MANT-ATP [150], and genetically encoded chimeric proteins of specific membrane/luminal vesicle markers and green fluorescence proteins (GFP) or mCherry-derived proteins [77, 79].

4.4.2.1 Secretory Vesicle Fusion in Astrocytes Occurs with a Delay upon

Stimulation—Compared to neurones it has been shown for all 4 secretory vesicles types described in astrocytes (Sect. 4.1) to fuse with the plasma membrane with a delay upon stimulation. As determined with C_m measurements the kinetics of secretory vesicle fusion in astrocytes is at least two orders of magnitude slower than that in neurons (Fig. 4.4b) [61], where secretory vesicle fusion occurs within <0.5 ms upon intracellular Ca^{2+} increase [131].

In respect to astroglial SLMVs the rise of cytosolic Ca^{2+} evoked by activation of metabotropic glutamatergic receptors [12, 19, 79] or purinergic receptors [119] triggers fusion events of SLMVs within hundreds of milliseconds after stimulation as determined in studies using fluorescently tagged VGluT1/2-containing vesicles (i.e., VGluT-pHluorin and VGluT-EGFP, which are chimeric proteins of VGluT and a pH-sensitive GFP protein ecliptic synapto-pHluorin (SpH; [83]) or EGFP. Ionomycin, a Ca^{2+} ionophore, triggers exocytotic fusion of SpH-labeled SLMVs within seconds [72]. In another study exocytotic bursts of SpH-labeled SLMVs occur within 6 s after mechanical stimulation of astrocytes, but other stimuli such as ATP, bradykinin, the Ca^{2+} ionophore 4-Br-A23187, α -latrotoxin, or hypertonicity cause fusion of SpH-labeled SLMVs following a delay of >1 min [77]. Secretion of astroglial peptidergic vesicles also occurs with a delay. Exocytosis of neuropeptide Y-positive peptidergic vesicles upon glutamate [113] or ionomycin stimulation [109] occurs with a delay of >1 min and exocytosis of emerald green-tagged AMP from peptidergic vesicles in 8-Br-cAMP-differentiated astrocytes occurred over a time scale of minutes upon ionomycin stimulation [94]. A similar time-course was observed, when exocytosis of FM-dye-labeled lysosomes was studied. FM-dye labeled lysosomes began to fuse with the plasma membrane with a delay of >1 min upon stimulation of astrocytes with Ca^{2+} ionophores A-23187 [71] and ionomycin, or upon ATP stimulation [150]. The exocytotic fusion of the majority of TI-VAMP positive quinacrine-labeled secretory vesicles [138], that likely represent secretory lysosomes, occurred with a delay of >2 min upon addition of different stimuli, including glutamate, ATP, ionomycin or upon stimulation with UV-induced Ca^{2+} uncaging [96, 111]. EGFP-LAMP1 (lysosomal-associated membrane protein 1)-labeled lysosomes and FITC-dextran-labeled lysosomes also undergo fusion with a delay of >40 s upon application of ionomycin [72], ATP and, a group I metabotropic glutamate receptor agonist (R/S)-3,5-dihydroxyphenylglycine [56]. Moreover, ectosomes carrying IL-1 β start to bleb and shed from astroglial plasma membrane with a 1–2 min delay upon ATP stimulation [13].

The reason for such a loose excitation-secretion coupling in astrocytes [136] may be that (i) the major source of Ca^{2+} in astrocytes is not the extracellular space as in neurones, but intracellular IP_3 -sensitive Ca^{2+} -storage organelles, which release Ca^{2+} only upon activation of receptor-mediated intracellular signalling cascades and production of IP_3 , (ii) the lack of active zones in astrocytes and slower delivery of secretory vesicles to the plasma membrane fusion sites upon stimulus application compared to neurons, where there are active zones [108], or (iii) differences in exocytotic vesicle fusion machinery between astrocytes and neurons (Fig. 4.4) with astroglial machinery exhibiting a slower vesicle fusion dynamics compared to neuronal exocytotic machinery [85].

4.4.2.2 Modes of Astroglial Secretory Vesicles Fusion—Exocytotic fusion of secretory vesicles in astrocytes exists in two major forms [23], as observed in neurones and neuroendocrine cells [46]. Namely, amperometric studies revealed that dopamine-loaded astrocytic vesicles fuse with the plasma membrane either by transient (kiss-and-run) exocytosis, with vesicle content only partially released, or by full-fusion exocytosis [23]. In optical studies in which the exocytosis of SLMVs expressing spH [14, 77] or SLMVs co-expressing VGluT1-mCherry/VGluT1-pHluorin [79] was studied, both modes, the transient and the full-fusion, of SLMV exocytosis were shown to occur in the same astrocyte simultaneously under spontaneous or stimulated conditions. 50–60% of all spontaneous exocytotic events were the full-fusion events, while 40–50% were the transient fusion events. Depending on the type of a stimulus, the percentage of either type of event shifted toward transient or full-fusion modes of exocytosis upon stimulation. This indicates stimulus-dependent regulation of fusion pore opening [14, 23, 77]. Secretory lysosomes can also exhibit both transient and full-fusion modes of exocytosis. A rapid, total release of an FM dye was observed, followed by a slower and complete loss of EGFP-sialin (a lysosomal sialic acid transporter), from the same lysosomes upon mechanical stimulation, suggesting that secretory lysosomal fusion in astrocytes completes upon mechanical stimulation within seconds, without evidence for transient fusion [71]. Upon glutamate and ATP stimulation the release of FM dyes and MANT-ATP from LAMP1-positive lysosomes is only partial, implying the transient mode of secretory lysosomal exocytosis [150]. Vesicular nucleotide transporter mCherry has been shown to remain associated with the lysosomal membrane during the release of cathepsin D-Venus from the same lysosomes upon ATP, L-glutamate, and calcium ionophore A23187 stimulation, further suggesting that secretory lysosomes in astrocytes may not fully fuse with the plasma membrane [93]. Discrete increases in membrane capacitance, indicating single-vesicle fusion, revealed that astrocyte stimulation increases the frequency of predominantly transient fusion events in smaller vesicles (likely SLMVs and peptidergic vesicles), whereas larger vesicles (likely secretory lysosomes) transitioned to full fusion suggesting that vesicles with different diameters in astrocytes exhibit different capacities to discharge their cargo, due to distinct fusion pore properties [39].

The underlying molecular mechanisms controlling the fusion pore state are not clearly known, but may among others involve SNARE proteins and dynamin. Dynamin, a multidomain GTPase involved in vesicle scission from the plasmalemma during endocytosis, has been shown to be involved in the regulation of a fusion pore during spontaneous exocytosis in astrocytes, since activators of dynamin RyngoTM–1–23 promoted fusion pore closure by prolonging closed and by shortening open fusion pore dwell times [66]. DnSNARE (dominant-negative domain of synaptobrevin 2 protein) peptide, which interferes with endogenous VAMP2 expression and thus prevents VAMP2-mediated membrane fusion, has been shown to stabilize the fusion-pore diameter to narrow, release-unproductive diameters regardless of vesicle diameter, implying the regulatory role of SNAREs in governing vesicle fusion in astrocytes [39]. The fusion pore can alone be a subject of regulation by ketamine, an anesthetic that exhibits analgesic, psychotomimetic, and rapid antidepressant effects. It has been shown recently, using high-resolution cell-attached membrane capacitance measurements, that ketamine evokes long-lasting flickering of a

narrow fusion pore that is incapable of transiting to full fission [65]. Furthermore, ketamine treatment also suppressed ATP-triggered vesicle fusion and BDNF secretion by increasing the probability of a narrow fusion pore open state and/or by reducing astrocytic Ca^{2+} excitability [130].

4.5 Non-vesicular Astroglial Secretion

Astrocytes can also release signalling molecules by a non-vesicle-based mechanisms (i) through plasmalemmal channels (e.g., volume-regulated anion channels, connexons/pannexons (hemichannels), ionotropic pore-forming P2X_7 purinergic receptors, the two-pore-domain potassium channel Trek-1, or Bestrophin-1 channels [146], and (ii) through plasmalemmal transporters (e.g., reversal uptake by plasma membrane excitatory amino acid (glutamate) transporters, (hetero)exchange via the cystine–glutamate antiporter or organic anion transporters) [76]. With the exception of Bestrophin-1, these non-vesicular release mechanisms are Ca^{2+} -independent and might be activated only under pathological conditions [2, 45].

4.6 Concluding Remarks

Astrocytes are involved in many processes in the CNS through sensing extracellular signaling molecules by surface GPCRs, responding to this with cytosolic excitation, which then stimulates the release of their own astroglial chemical messengers, gliosignaling molecules. Many studies support the existence of vesicle-based secretion of transmitters from astrocytes, in response to GPCR-mediated stimulation. These studies have shown that astrocytes possess various types of secretory vesicles. The exocytotic fusion of these vesicles is regulated at the level of a single fusion pore and it occurs in two modes, as transient and full-fusion exocytosis. Moreover, astrocytes, which are electrical silent, but exhibit GPCR-mediated cytosolic excitability, respond to stimulation with a delay in exocytosis compared to fast responsive electrically excitable neurones. Such slow release kinetics of vesicle signaling apparatus suggests that astrocytes are acting as integrators of information, modulating neuronal activity in a slow-time domain. Although the physiological relevance of astroglial exocytosis in vivo is still not clear, it is predicted that astrocytes participate in information processing in the brain by exocytotic release of signaling molecules.

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Fig. 4.1.

The vesicle network in astrocytes. Lysosomes, first described in 1955, represent a central, prominent intermediate of endo- and exocytotic pathways in all eukaryotic cells, including astroglia. Intracellular secretory organelles (synaptic-like vesicles, dense-core vesicles and primary lysosomes) originate from the endoplasmic reticulum and Golgi complex. Primary lysosomes fuse with endosomes, phagosomes and autophagosomes and convert to secondary lysosomes that undergo exocytosis, thus expelling products of degradation. The multivesicular bodies contain exosomes that may carry various signaling factors. Modified with permission [152]

Fig. 4.2.

Secretory vesicles studied by STED and SIM microscopies in acutely isolated rat astrocytes.

a Confocal and STED microscopy images of immunostained vesicles D-serine-, V-GLUT1-, ANP- and BDNF-positive vesicles in acutely isolated astrocytes. Histograms display STED-acquired vesicle diameter distributions for 1788 (D-serine), 6787 (V-GLUT1), 1747 (ANP) and 798 (BDNF) vesicles (2 cells per staining). The black curves show Gaussian fits of the diameter distributions; the numbers next to the distribution peaks indicate the average vesicle diameter (expectation value $\pm$ SEM). Recalculated values taking into account the microscope's optical resolution (45 nm) are 80.8 nm for D-serine, 88.4 nm for V-GLUT1, 85.9 nm for ANP and 86.8 nm for BDNF. Scale bar, 500 nm. **b** Wide-field microscopy and SIM were used to determine the vesicle diameter of immunostained LAMP1 endolysosomes and ATP-loaded vesicles (quinacrine dihydrochloride). Histograms show SIM-acquired vesicle diameter distributions for 557 (LAMP1, 2 cells) and 445 (quinacrine, 2 cells) vesicles in acutely isolated astrocytes (upper two panels) and 338 (LAMP1, 3 cells) and 333 (quinacrine, 6 cells) vesicles in astrocytes isolated from 7- to 8-week-old rats (lower two panels). The black curves show Gaussian fits of the diameter distributions; the average vesicle diameter (expectation value $\pm$ SEM) is labeled next to the distribution peaks. Scale bar, 500 nm. Modified with permission [39]

Fig. 4.3.

Internalization of exosomes into astrocytes. Internalization of PKH26 nanoparticles and PKH26-positive particles of the exosome-containing samples into subcellular compartments of cultured astrocytes. **a, b** Representative three-dimensional shaded display of individual live cultured astrocytes that internalized PKH26 nanoparticles (**a**, con) and PKH26-positive particles present in the PKH26-labeled exosome-containing samples (**b**, exo) into intracellular compartments, observed as numerous bright fluorescent puncta. Scale bars, 10 µm. Modified with permission [112]

Fig. 4.4.

Slowness of astroglial exocytosis. **a** Neuronal versus astrocytic SNAREs. Neurones and astrocytes alike express SNAREs VAMP2 and syntaxin 1; many astrocytes can also express VAMP3 in lieu of or in addition to VAMP2. Astrocytes express SNAP23, a homologue of neuronal SNAP25. At the plasma membrane, syntaxin 1A can form a binary cis complex with SNAP25B or SNAP23A, which then interacts with vesicular VAMP2 to form a ternary complex. A single ternary complex can tether the vesicle at the plasma membrane for a longer period of time, when it contains SNAP25B rather than SNAP23A, respectively. Of note, truncated syntaxin 1, lacking the N-terminal Habc domain and the linker region to the

SNARE domain, is shown for simplicity. Drawings are not to scale. **b** Comparison of kinetics of neuronal and astroglial exocytosis. Time-dependent changes in membrane capacitance (C_m) recorded in a neuronal cell (trace in red, photoreceptor) and an astrocyte (trace in blue), elicited by a flash photolysis-induced increase in cytosolic Ca^{2+} . Note that the blue trace recorded in an astrocyte displays a significant delay between the stimulus (asterisk) and the response (trace components above the dotted line). Modified with permission [139]