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REVIEW ARTICLE

Aspects of astrocytic cAMP signaling with an emphasis on the putative power of compartmentalized signals in health and disease

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Abstract

This review discusses aspects of known and putative compartmentalized 3′,5′-cyclic adenosine monophosphate (cAMP) signaling in astrocytes, a cell type that has turned out to be a key player in brain physiology and pathology. cAMP has attracted less attention than Ca^{2+} in recent years, but could turn out to rival Ca^{2+} in its potential to drive cellular functions and responses to intra— and extracellular cues. Further, Ca^{2+} and cAMP are known to engage in extensive crosstalk and cAMP signals often take place within subcellular compartments revolving around multi-protein signaling complexes; however, we know surprisingly little about this in astrocytes. Here, we review aspects of astrocytic cAMP signaling, provide arguments for an increased interest in this subject, suggest possible future research directions within the field, and discuss putative drug targets.

KEYWORDS
adenylyl cyclase, AKAP, astrocyte, biosensor, Ca^{2+}, cAMP, signaling

1 | INTRODUCTION

Astrocytes are intimately coupled to neuronal function via bidirectional intercellular signaling and exchange of metabolites, and astrocytes have turned out to be key cells in brain physiology and pathology (Schousboe, Bak, Madsen, & Waagepetersen, 2012; Verkhratsky & Nedergaard, 2018). Astrocytic Ca^{2+} signaling has been studied extensively for decades whereas the other "canonical" second messenger, 3′,5′-cyclic adenosine monophosphate (cAMP), has attracted somewhat less attention. We started taking an interest in cAMP signaling in astrocytes when we discovered that crosstalk between Ca^{2+} and cAMP signals is involved in glycogen dynamics (Müller, Fox, Schousboe, Waagepetersen, & Bak, 2014) which led us to engage in several projects to further investigate this. While aspects of receptor-mediated astrocytic cAMP signaling was recently reviewed by Horvat and Vardjan (2019) and Zhou, Ikegaya, and Koyama (2019), the raison d'être for this review is to further appreciate the putative compartmentalization of the cAMP signals that are driven both directly and indirectly by receptor-coupled pathways, and to motivate an increased research interest in astrocytic cAMP signals.

ABBREVIATIONS: AC, adenylyl cyclase; AKAP, A-kinase anchoring protein; AMPK, 5′ adenosine monophosphate-activated protein kinase; CaM, calmodulin; cAMP, 3′,5′-cyclic adenosine monophosphate; CaN, calcineurin; CFP, cyan fluorescent protein; DREADD, designer receptor exclusively activated by designer drugs; Epac, exchange proteins directly activated by cAMP; ER, endoplasmic reticulum; FRET, fluorescence resonance energy transfer; GPCR, G protein-coupled receptor; IP_3, inositol 1,4,5-triphosphate; Orai, calcium release-activated calcium channel protein; GS, glycogen synthetase; GP, glycogen phosphorylase; PDE, phosphodiesterase; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; sAC, soluble adenylyl cyclase; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; SOCE, store-operated calcium entry; tmAC, transmembrane bound adenylyl cyclase; TRP, transient receptor potential channel; TRPV1, transient receptor potential channel V (vanilloid) 1; YFP, yellow fluorescent protein.

2 | POTENTIAL COMPARTMENTALIZATION OF cAMP SIGNALS

Cyclic AMP is a ubiquitous second-messenger discovered by Earl Sutherland who was awarded the 1971 Nobel Prize in Physiology or Medicine for his discovery. The initial idea for this work came from earlier work on glycogen metabolism in the lab of Nobel laureates Gerty and Carl Cori, and glycogen metabolism remains the prime example of cAMP-mediated effects in biochemistry textbooks. However, cAMP

signaling play a multitude of roles in cells, and interacts with other signaling pathways including Ca^{2+}-mediated signaling (Figure 1). As recently pointed out by Bazargani and Attwell (2016) there are likely many discoveries waiting to be made regarding cAMP signaling in relation to astrocytes, as has been the case for the Ca^{2+} signaling field over the last couple of decades. So why is that? We know that Ca^{2+} has many targets in the cell, whereas cAMP seems to target only four effector protein families: protein kinase A (PKA), exchange protein directly activated by cAMP (Epac), cyclic nucleotide-gated ion channels, and the family of Popeye-domain containing proteins (Cooper & Tabbasum, 2014). So how may cAMP signaling rival Ca^{2+} signaling in complexity and diversity? As discussed by Cooper and Tabbasum (2014), one answer is that, as for many types of Ca^{2+} signaling events, the cAMP signal may be restricted to microdomains (or even nanodomains) within the cell; the adenylyl cyclase (AC) generating the cAMP signal, PKA, and the enzyme degrading cAMP, phosphodiesterase (PDE), may be located in a multi protein complex held together by members of a group of scaffold proteins known as A-kinase anchoring proteins (AKAPs; Figure 2; Cooper, 2005; Dessauer, 2009). In such a complex, cAMP may potentially be produced, exert its effect, and be degraded within a well-defined part of the cell (Cooper & Tabbasum, 2014). However, in many studies, cAMP signals can be detected throughout the cell in response to receptor agonists, suggesting that not all signals are compartmentalized or that initially compartmentalized signals can spill into the main cytosolic compartment. As a clear example of compartmentalization, β1 or β2 adrenoceptor (AR) activation in cardiac myocytes led to distinct cellular distribution of the cAMP signals which correlated with discrete engagement of downstream effectors (as discussed by

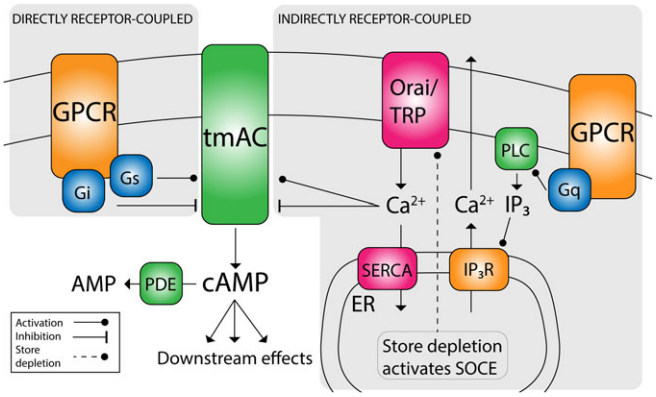

FIGURE 1 Directly and indirectly receptor-coupled cAMP signaling. The formation of cAMP can be regulated in two ways: The enzyme transmembrane adenylyl cyclase (tmAC) is activated or inhibited either directly by receptor-coupling through the G proteins, G_s and G_i, or indirectly via store-operated Ca^{2+} entry (SOCE). SOCE occurs when endoplasmic reticulum (ER) Ca^{2+} concentration falls below a certain threshold which then activates Ca^{2+} uptake by store-operated plasma membrane calcium channels named Orai or transient receptor potential (TRP) channel. This might happen secondary to G protein-coupled receptor (GPCR) Gq activation where inositol triphosphate (IP_3), generated by phospholipase C (PLC), causes a fall in ER Ca^{2+} stores via IP_3 -receptor (IP_3R) activation. The cAMP formed acts on downstream effectors and is continuously degraded by phosphodiesterases (PDEs) to AMP

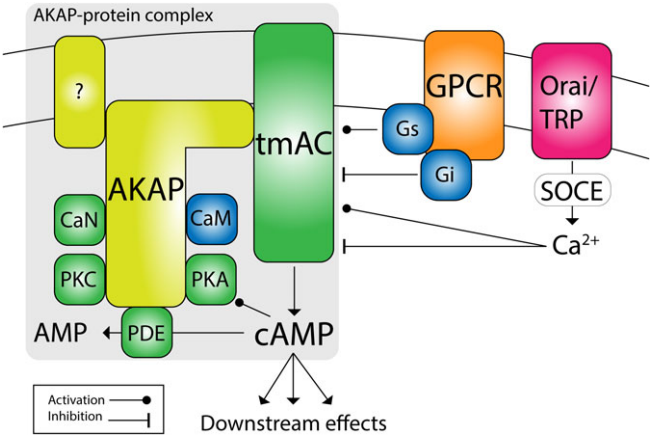

FIGURE 2 A-kinase anchoring proteins (AKAPs) facilitate and regulate the spatial and temporal dynamics of cAMP signaling. Transmembrane-bound adenylyl cyclases (tmACs) are activated or inhibited, directly or indirectly by Ca^{2+} or G protein-coupled receptor (GPCR) activation. tmACs are known to bind to AKAPs, which act as scaffold proteins in protein complexes. They always bind protein kinase A (PKA) and may bind phosphodiesterases (PDEs) allowing tight spatial and temporal regulation of cAMP dynamics. cAMP may then both be formed, work, and degraded in such protein complexes or diffuse to other downstream effectors. AKAPs can also bind calmodulin (CaM), calcineurin (CaN), and protein kinase C (PKC); all proteins involved in Ca^{2+} signaling, representing another entry point for the interplay between Ca^{2+} and cAMP signaling. Membrane-bound receptors and ion channels (indicated by a question mark), such as GPCR and L-type Ca^{2+} channels have also been observed to participate in such AKAP complexes (Wild & Dell'Acqua, 2018) raising the question whether they are involved in cAMP dynamics in astrocytes

Ellisdon & Halls, 2016; initial observation by Nikolaev, Bunemann, Schmitteckert, Lohse, & Engelhardt, 2006); this clearly demonstrates that compartmentalization of cAMP signals are functionally important in cells as first suggested by Buxton & Brunton (1983). Utilizing targeted cAMP sensors, the cAMP signal was found to display different signaling dynamics dependent on whether cAMP was measured in the cytosol, at the membrane or near lipid rafts (Agarwal et al., 2014; DiPilato, Cheng, & Zhang, 2004); however, for all types of stimulation, the cAMP response could be measured by all the targeted sensors. This suggests that while some degree of cAMP compartmentalization occurs, it most likely does not exclusively explain the separate responses observed when using different G protein-coupled receptor (GPCR) agonists. Extending on the concept of compartmentalization, the intracellular diffusion of cAMP has been shown to occur at a rate substantially slower than in aqueous solution (Agarwal et al., 2014). Clearly, cAMP will bind to its effector proteins throughout the cell and in this respect, it is noteworthy that the regulatory subunit of the main effector of cAMP, PKA, is an abundant protein and outnumbers the catalytic subunit in a wide range of tissues including brain (Walker-Gray, Stengel, & Gold, 2017); incidentally, these authors also make a solid case for the idea that the catalytic subunit dissociates from the regulatory subunit upon binding of cAMP, a long-held notion that was recently challenged (Smith et al., 2013; Smith et al., 2017). On a final note on PKA's contribution to compartmentalization of cAMP-mediated

responses, the findings of Walker-Gray et al. also suggest that active PKA catalytic subunits do not travel far before they are recaptured by regulatory subunits (Walker-Gray et al., 2017). In conclusion, spatially and functionally distinct cAMP signals may be generated within the same cell at any given time as a consequence of one or more extracellular cues or indeed, as we will discuss below, in response to an intracellular cue.

3 | POTENTIAL COMPLEXITY OF cAMP SIGNALING IN ASTROCYTES

Excellent detailed reviews on the molecular players of cAMP signaling have been published (e.g., Cooper & Tabbasum, 2014; Dessauer et al., 2017; Kamenetsky et al., 2006), so we will confine this section to a short outline of the key players and concepts of importance for the subsequent discussion. cAMP is synthesized from ATP by the enzyme AC of which 10 distinct isoforms have been identified (Kamenetsky et al., 2006). Nine of these are plasma transmembrane bound (tmACs) and most seem to respond to GPCR signaling via activation or inhibition by Gαs or Gαi subunits, respectively (Kamenetsky et al., 2006). A cytosolic variant called soluble AC (sAC) exists as well and is activated by HCO_3^- and micromolar levels of Ca^{2+}; it should be noted though that sAC is not freely diffusing in the cytosol but rather exists in discrete microdomains within the cell including several organelles (Kamenetsky et al., 2006; Litvin, Kamenetsky, Zarifyan, Buck, & Levin, 2003). Transcriptomic and proteomic studies of mouse brain cells revealed that all the tmACs except tmAC isoform 4 (AC4) are found in astrocytes and indicated the presence of sAC (Sharma et al., 2015; Zhang et al., 2014a). However, the proteomic study did not find sAC in any brain cell type, which is in contrast to several studies showing the functional presence of sAC in cultured astrocytes and brain mitochondria (Acin-Perez et al., 2009; Acin-Perez et al., 2011; Choi et al., 2012; Jakobsen et al., 2018; Jakobsen et al., 2017b).

Despite similarities, the nine tmACs are regulated differently by GPCRs, Ca^{2+}/calmodulin (CaM), and a variety of kinases, which allows for highly specific cAMP signals to be involved in distinct signaling pathways (Cooper & Tabbasum, 2014). Interestingly, some tmACs operate in the absence of interactions with GPCRs being either activated or inhibited by Ca^{2+} directly or indirectly (Halls & Cooper, 2011; see Figure 1). This concept is key to understand the complexity of cAMP signaling, and how it interacts laterally with Ca^{2+} signaling pathways to modulate the cAMP signal in complex ways. Thus, a cAMP signal may be generated in the absence of a receptor-coupled primary signal—an important but often overlooked aspect of cAMP signaling. For instance, AC8 is activated by Ca^{2+} via CaM and has been shown to bind to Orai1 channels involved in store-operated Ca^{2+} entry (SOCE), a process activated by the depletion of the endoplasmic reticulum (ER) Ca^{2+} store. In fact, AC8 seems to only respond to Ca^{2+} moving into the cell via channels in the plasma membrane (Willoughby et al., 2012). Depletion of the ER Ca^{2+} store is of course a consequence of either inositol 1,4,5-trisphosphate (IP_3) or ryanodine receptor-mediated elevations of cytosolic [Ca^{2+}] with the latter mechanism being present at least in cultured astrocytes (Müller, Obel, Waagepetersen, Schousboe, & Bak, 2013). The biological significance of these Ca^{2+}-induced cAMP signals is not clear; however, in astrocytes we have suggested that it may be inducing breakdown of glycogen to support cytosolic Ca^{2+} homeostasis by powering Ca^{2+} uptake into the ER (Müller et al., 2014). One step toward fully understanding the complexity of astrocytic cAMP signaling is to pinpoint specific ACs to the observed cellular cAMP responses and downstream effects, as exemplified by AC8 in the above. Another component to the multifaceted regulation of the different tmAC isoforms is the interactions of tmACs with AKAPs. They are defined by their ability to bind PKA and all tmAC isoforms, except AC4 and 7, have been shown to participate in signaling hubs with at least one of these (Table 1). AKAPs are central to both spatial and temporal regulation of cAMP signaling, as they can bind both tmACs and effectors downstream of cAMP signaling (Efendiev, Bavencoffe, Hu, Zhu, & Dessauer, 2013). Some AKAPs have also been shown to bind PDEs facilitating a highly localized cAMP signal, as the cAMP can be degraded continuously close to where it was generated (Bauman et al., 2006; Figure 2). In the case of some AKAP-centered signaling hubs, PKA, or PKC interacting with the AKAP can modulate the cAMP production by phosphorylating the tmAC it associates with (Shen & Cooper, 2013; Willoughby et al., 2012). Such AC-AKAP complexes have not yet been described in any considerable details for astrocytes; however, numerous interactions could be possible based on which tmACs and which AKAPs have been shown to be expressed in astrocytes (Table 1; Horvat & Vardjan, 2019). These interactions could play a central role in astrocyte cAMP signaling outcomes, as has been shown to be the case for other cell types (Baldwin & Dessauer, 2018). These AC-AKAP complexes also represent the molecular basis for putative nanodomain cAMP signaling. A key example of this is the attenuating role of cAMP on desensitization of the transient receptor potential vanilloid 1 (TRPV1) channel in dorsal root ganglia from rats (Efendiev et al., 2013). This attenuation is dependent on the PKA anchored on AKAP150 (the murine variant of AKAP79; Zhang, Li, & McNaughton, 2008), and on the interactions of AKAP150 with TRPV1 channels and AC5 (Efendiev et al., 2013), suggesting that intact organization of such nanodomain complexes are important for the signaling outcomes of cAMP. Both AKAP79 and AC5 are expressed in astrocytes and could participate in a similar complex there. That AKAPs do likely play an important role in astrocyte biology and pathology was recently demonstrated by Rivera-Pagan et al. (2018) who showed that knock down of AKAP150 depolarizes the astrocytic plasma membrane and reduces their ability to clear extracellular K^+ during ischemic conditions.

4 | METHODS TO STUDY cAMP SIGNALING DYNAMICS

cAMP is an organic molecule and may be quantified by different means. Common commercial chemical and radioimmuno-based assays are all endpoint measurements, and thus will not inform the investigator about the spatiotemporal dynamics of the cAMP signal in intact

TABLE 1 RNA-sequencing data for expression of AKAPs in mouse brain astrocytes and their known associated tmAC isoforms in various cell types

Gene name	Protein names and isoforms	Relative expression levels in astrocytes		Known to participate in cAMP signaling complexes with tmACs
		Brain cortex FPKM[a]	Total brain RPKM[b]	
AKAP1	D-AKAP, s-AKAP84, AKAP121, AKAP149	6.52	1.88	
AKAP2	AKAP-KL	4.49	29.82	
AKAP5	AKAP79 (human), AKAP75 (bovine), AKAP150 (murine)	1.05	0.25	AC1-3, 5, 6, 8, 9[c]
AKAP6	mAKAPβ	7.76	1.04	AC5[c]
AKAP7	AKAP15, AKAP18 (α, β, γ, δ)	18.03	4.55	
AKAP8	AKAP95	28.81	5.39	
AKAP9	Yotiao, AKAP450, AKAP350, CG-NAP, Hyperion	9.51	1.52	AC1-3, 9[c]
AKAP10	D-AKAP2	6.43	1.51	AC5[d]
AKAP11	AKAP220	13.72	11.7	
AKAP12	Gravin, SSeCKS	4.97	36.72	
AKAP13	AKAP-Lbc, Ht31, BRX-1	5.52	1.42	
ARFGEF2	BIG2	7.12	1.73	
EZR	Ezrin	118.02	117.62	
MAP2	MAP2B	63.62	51.32	AC1, 8[e]
CMYA5	Myospryn	0.98	0.11	
SPHKAP	SKIP	0.15	0.04	
SYNM	Synemin	1.72	2.38	
TNNT2	Troponin T	0.1	0.15	
LDB3	Cypher, ZASP	0.2	0.2	
C2orf88	smAKAP	0.53	0.29	
PCNT	Pericentrin	2.74	5.36	AC3[f]
WASF-1	Wave-1	11.25	5.28	
ACBD3	PAP7	12.79	10.13	
NBEA	Neurobeachin	4.89	1.04	
AKAP14	Akap28	0.12	4.2	
CBFA2T3	Myeloid translocation gene (MTG) 8 and 16b	0.1	0.38	
RAB32	Rab32	0.73	24.21	
MYRIP	Myosin-VIIa- and Rab-interacting protein	6.86	0.08	
NF2	Merlin	13.47	1.35	
AKAP17A	SFRS17A	Not in database		
AKAP8l	HAP95	27.46	3.16	
GSKIP	GSK3B-interacting protein	3.27	38	

Note. This list is not exhaustive, and where no tmAC participation is listed, this does not preclude the possibility.

Abbreviations: FPKM, fragments per kilobase of transcript sequence per million mapped fragments; RPKM, reads per kilobase of exon per million mapped fragments; tmAC, transmembrane adenylyl cyclase.

[a]Data from Zhang et al., 2014a.

[b]Averaged from Sharma et al., 2015.

[c]Reviewed in Cooper & Tabbasum, 2014.

[d]Guinzberg et al., 2017.

[e]Conti et al., 2007.

[f]Guadiana et al., 2016.

cells and tissues. The introduction of fluorescent protein biosensors that can be expressed in cells revolutionized the field since they allow the investigator to monitor real-time changes in the cellular cAMP signal employing fluorescence microscopy or plate reader-based assays that may be adapted for high throughput (Mathiesen, Vedel, & Brauner-Osborne, 2013; Vedel, Brauner-Osborne, & Mathiesen, 2015; Willoughby & Cooper, 2008). See Figure 3 for a timeline of the development of selected techniques for measuring cAMP and Ca^{2+}. The available biosensors for cAMP are based on the fluorescence resonance energy transfer (FRET) principle employing parts of one of the two cAMP-binding proteins, PKA or Epac, as the central cAMP-binding entity flanked by a pair of fluorescent proteins such as CFP/YFP (e.g., Bacskai et al., 1993; Dou et al., 2015; Everett & Cooper, 2013; Halls, 2018; Hempel, Vincent, Adams, Tsien, & Selverston, 1996; Nikolaev et al., 2004; Zaccolo et al., 2000; Zaccolo & Pozzan, 2002). The advantages and limitations of such biosensors have been reviewed in detail elsewhere (Halls, 2018; Willoughby & Cooper, 2008). However, it is important to stress that the investigator should pay attention to the compartmentalization of the cAMP signal as well as the technical issues related to measuring cAMP signals employing the different available biosensors. Thus, sensitivity of the biosensor to changes in pH needs to be considered, as does the correct subcellular targeting because bulk (cytosolic) and for example, plasma membrane-localized cAMP signals may not be equivalent, and thus erroneous conclusions may be drawn (e.g., Dyachok et al., 2008; Everett & Cooper, 2012; Everett & Cooper, 2013). For Ca^{2+}, the importance of membrane-targeting was initially shown by employing a plasma membrane-targeted biosensor to study Ca^{2+} dynamics in astrocyte processes rather than cell body responses typically reported when employing dyes and non-targeted biosensors (Shigetomi, Kracun, Sofroniew, & Khakh, 2010). Clearly, in terms of interacting with its surroundings, the functionally important part of an astrocyte is not the cell body but the processes interacting with neurons and blood vessels. Another important technical issue is the fact that biosensors based on PKA are less well suited to report cAMP dynamics than Epac-based biosensors, since PKA has four binding sites for cAMP with different affinities. Also, PKA is a powerful initiator of signaling pathways that makes the use of a PKA-based biosensor problematic unless one wants to investigate activation of PKA rather than the cAMP signal per se employing A-kinase activity reporters (Halls, 2018). As for Ca^{2+} biosensors, transgenic animals

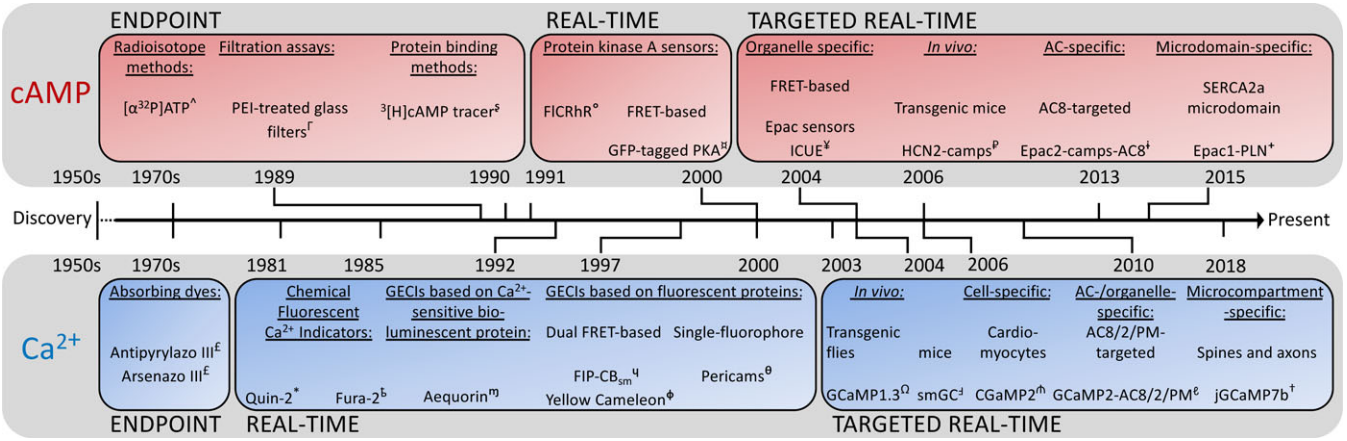

FIGURE 3 Development of intracellular cAMP and Ca^{2+} measurement tools. Since the discoveries of cAMP and Ca^{2+} as second messengers, various methods to investigate changes in intracellular concentrations have been employed. Initially, all methods relied on endpoint measurements, which posed challenges in studying both kinetics and compartmentalization. In the 1980s, the development of the ratiometrical chemical fluorescent Ca^{2+} indicators like quin-2 and fura-2 revolutionized the Ca^{2+} signaling field and made it possible to do fluorescent lifetime measurements—a decade before real-time measurement of cAMP was possible with the ratiometric reporter FlCRhR. The investigation of fluorescent resonance energy transfer (FRET)-based biosensors facilitated the direct visualization of the spatiotemporal dynamics of both messengers. To date, the repertoire of engineered genetically-encoded reporters are vast. Thus, specific targeted sensors or transgene animals are necessary to obtain an understanding of subcellular compartmentalization of cAMP and Ca^{2+} on the nanometer scale. This timeline gives an overview of the cAMP and Ca^{2+}methodological progresses from discovery to present including some representative references. Data for timeline as reported in literature (for detailed cAMP reporters see Jiang, Falcone, Curci, and Hofer (2017); Musheshe, Schmidt, and Zaccolo (2018); Paramonov, Mamaeva, Sahlgren, and Rivero-Muller (2015); Schleicher and Zaccolo (2018)) (for Ca2+ see Campbell (2015); Grienberger and Konnerth (2012); Looger and Griesbeck (2012); Suzuki, Kanemaru, and Iino (2016)). ^, Salomon, Londos, and Rodbell (1974); ⌜, Takeda, Kuno, Shuntoh, and Tanaka (1989); $, Nordstedt and Fredholm (1990); °, Adams, Harootunian, Buechler, Taylor, and Tsien (1991); ¤, Zaccolo et al. (2000); Zaccolo and Pozzan (2000); ¥, DiPilato et al. (2004); Nikolaev, Bunemann, Hein, Hannawacker, and Lohse (2004); Ponsioen et al. (2004); ℗, Nikolaev et al. (2006)ⁱ, Everett and Cooper (2013); +, Sprenger et al. (2015); £, Vergara and Delay (1985); *, Grynkiewicz, Poenie, and Tsien (1985); ♭, Tsien, Rink, and Poenie (1985); ♍, Kendall, Sala-Newby, Ghalaut, Dormer, and Campbell (1992); ♄, Romoser, Hinkle, and Persechini (1997); ♣, Miyawaki et al. (1997); ♓, Nakai, Ohkura, and Imoto (2001); Sawano and Miyawaki (2000); Ω, Wang, Wong, Flores, Vosshall, and Axel (2003); ♩, Ji et al. (2004); ♌, Tallini et al. (2006); ℘, Willoughby, Wachten, Masada, and Cooper (2010); †, Dana et al. (2018). Abbreviations: AC, adenylyl cyclase; Epac, exchange protein directly activated by cAMP; GECI, genetically encoded Ca^{2+} indicators; HCN2, hyperpolarization-activated cyclic nucleotide-gated channel 2; PEI, polyethyleneimine; PKA, protein kinase A; PLN, phospholamban; PM, plasma membrane; smGC, smooth muscle isoform of myosin heavy chain-GCaMP1 expressing mice

expressing a targeted cAMP biosensor have been produced and successfully employed to study a cAMP microdomain in proximity to SERCA pumps in cardiac myocytes (Sprenger et al., 2015). Thus, the real-time imaging of cAMP is now more an issue about which targeted sensor is employed and which subcellular organization of the multifunctional cAMP signaling pathway wished to be investigated (Musheshe et al., 2018, Surdo et al., 2017).

5 | MULTIPLE ROLES OF cAMP SIGNALING IN ASTROCYTES

cAMP signaling plays a multitude of roles in cells, and astrocytes are no exception. In the following, we will limit the discussion to three well-described subjects, namely glycogen metabolism, dynamic changes in morphology, and learning and memory.

5.1 | Glycogen metabolism

Regulation of glycogen metabolism is the prototypical example of cAMP/PKA-mediated signaling in many biochemistry textbooks. For detailed reviews on the Ca^{2+} and cAMP-mediated regulation of glycogen metabolism including both receptor and non-receptor mediated aspects, please see Bak, Walls, Schousboe, and Waagepetersen (2018) and Obel et al. (2012). The enzyme phosphorylase kinase, which activates the glycogen-degrading enzyme glycogen phosphorylase (GP) via phosphorylation, is activated by Ca^{2+} directly (positive allosteric modulator) and by PKA-mediated phosphorylation (Figure 4). The relative importance of Ca^{2+}- and cAMP/PKA-mediated engagement of glycogen breakdown is not entirely clear and somewhat divergent data have been published (reviewed in Bak et al., 2018; Obel et al., 2012). Thus, primary signals causing secondary cAMP or Ca^{2+} signals may all trigger breakdown of glycogen although due to the compartmentalization of the signals (and possibly intracellular location of glycogen) it is unlikely that they all do. It should be mentioned that synthesis of glycogen by glycogen synthetase (GS) is reciprocally regulated in the sense that PKA-driven phosphorylation switches this enzyme off (Obel et al., 2012). Despite the reciprocal regulation of glycogen synthesis and degradation, a fraction of the glucose molecules entering the astrocyte are shunted through glycogen before undergoing glycolysis, suggesting simultaneous activity of GP and GS (Walls, Heimbürger, Bouman, Schousboe, & Waagepetersen, 2009). This phenomenon, called the glycogen shunt, is still not fully understood but its high activity underlines the significance of astrocytic glycogen metabolism. Interestingly, astrocytes express two different isozymes of GP, a brain (B) and a muscle form (M), and we addressed the individual regulation of these by phosphorylation showing that GPM primarily responds to phosphorylation whereas GPB primarily responds to allosteric activation by AMP (Müller, Pedersen, Walls, Waagepetersen, & Bak, 2015; Figure 4). Recently, we linked the muscle isoform of GP to the glycogen shunt, which indicates that one cAMP signal activates GP while another cAMP signal is being suppressed to allow GS activity (Jakobsen et al., 2017a). While

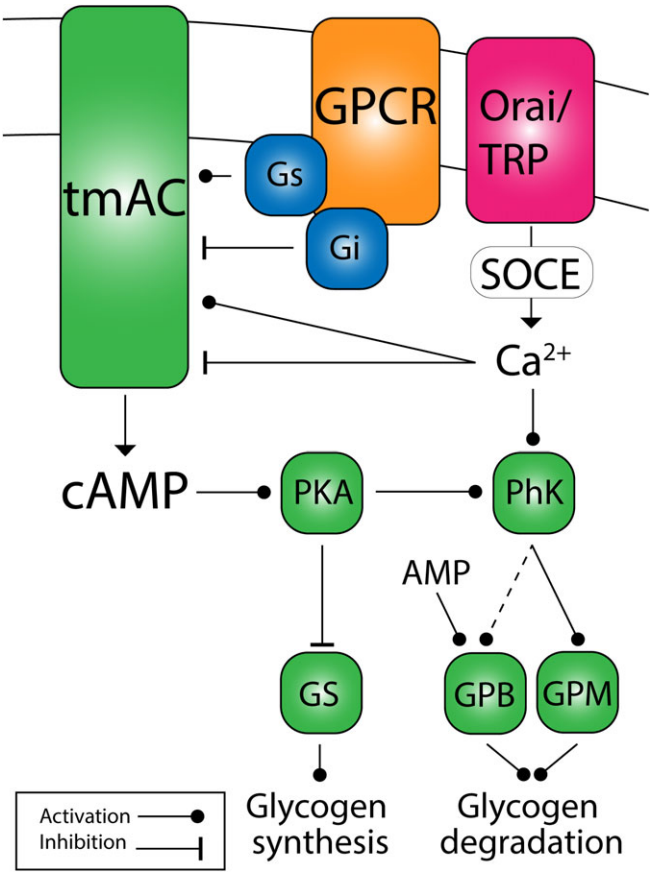

FIGURE 4 cAMP and Ca^{2+}-mediated regulation of glycogen metabolism in astrocytes. Extracellular signals activate transmembrane-bound enzyme adenylyl cyclases (tmACs) to generate the formation of cyclic AMP (cAMP). This happens either directly via G protein-coupled receptor (GPCR) activation or indirectly via store-operated Ca^{2+} entry (SOCE), raising intracellular [Ca^{2+}] by store-operated plasma membrane calcium channels named Orai or transient receptor potential (TRP) channel. The cAMP formed act on downstream effector families, such as protein kinase A (PKA). This inhibits the synthesis of glycogen by phosphorylation of glycogen synthetase (GS). Reciprocally, PKA-driven phosphorylation or Ca^{2+} binding (positive allosteric modulator) of the enzyme phosphorylase kinase (PhK) activates the glycogen-degrading enzyme, glycogen phosphorylase (GP), which exist in two isoforms in astrocytes: a brain (B) and a muscle form (M). The GPM isoform primarily responds to phosphorylation, whereas the GPB isoform primarily responds to allosteric activation by AMP leading to degradation of glycogen

glycogen degradation is often related to GPCR and subsequent tmAC activity, a non-receptor-mediated pathway involving sAC-produced cAMP has been suggested (Choi et al., 2012). In the model proposed by Choi et al., the elevation in extracellular K^+ produced by neuronal activity causes an increase in astrocytic HCO_3^- levels by uptake of K^+ and downstream activity of the Na^+/ HCO_3^- co-transporter. This increase in HCO_3^- was suggested to activate sAC and the subsequent cAMP response to lead to degradation of glycogen providing lactate to support the high energy demanding synaptic activity (for recent reviews on the role of lactate shuttling from astrocytes to neurons, please see Bak & Walls, 2018a; Bak & Walls, 2018b; Barros & Weber,

2018a; Barros & Weber, 2018b; Diaz-Garcia & Yellen, 2018). One potentially severe caveat in the study by Choi et al. (2012) is that they employed KH7, a selective inhibitor of sAC, in their key experiments. We recently showed that KH7 uncouples mitochondria causing a potential increase in AMP levels and AMPK activation (Jakobsen et al., 2018); thus, conclusions drawn from experiments employing KH7 should be interpreted with caution. Finally, as indicated above, an open question is whether all cAMP signals are equal in terms of activating the subset of PKA responsible for initiating glycogen breakdown. Thus, future research should address which receptor and especially non-receptor-coupled cAMP signals mediates glycogen breakdown.

5.2 | Learning and memory

Recent reviews highlight the involvement of astrocytes in memory formation for example, via changes in morphology (and thus synaptic contacts) and metabolism (Alberini, Cruz, Descalzi, Bessieres, & Gao, 2017; Gibbs, 2015b; Zorec, Horvat, Vardjan, & Verkhratsky, 2015), and we know that astrocyte glycogen dynamics is required for memory formation in animals (Duran, Saez, Gruart, Guinovart, & Delgado-Garcia, 2013; Gao et al., 2016; Gibbs & Hutchinson, 2012; Gibbs, Lloyd, Santa, & Hertz, 2007; Hertz & Gibbs, 2009; Hertz, O'Dowd, Ng, & Gibbs, 2003; Suzuki et al., 2011). We also know that Ca^{2+}-driven cAMP signals are central to learning and memory, since double knockout of AC1 and 8 (but, interestingly, not single knockouts) inhibits learning and memory in rodents (Wong et al., 1999); conversely, astrocytic adenosine A_{2A}-Gs-cAMP signaling impairs long-term memory formation in rodents (Orr et al., 2015). The latter work is discussed below in the context of dementia pathology. One article that clearly establishes the power of compartmentalized cAMP signaling events in learning and memory is the finding by Gao et al. (2016) that astrocytic β2ARs, but not β1ARs, are important for emotionally relevant memories via arousal-induced noradrenergic signaling from the locus coeruleus. Since the authors linked the finding to β2AR-mediated glycogenolysis and lactate release for neuronal consumption, this implies that β2ARs but not β1ARs have access to engage the glycogen pool. An interesting question here is which compartmentalized signaling pathways and downstream effectors the two receptors engage in astrocytes. As noted above, the two β receptors evoke clearly compartmentalized cAMP signals in cardiac myocytes (Nikolaev et al., 2006). On a final note, it should be pointed out that lactate transfer might not be the explanation for astrocytic support of synaptic plasticity but rather that the breakdown of glycogen in astrocytes spares extracellular/blood-borne glucose for neuronal use, as suggested recently by Dienel (2019).

5.3 | Morphology and maturation of astrocytes

The elevation of cAMP in cells is well known to have an enormous influence on the stellation, that is, the morphological differentiation of cells. Already in the 70s, Moonen and Sensenbrenner (1976) employed dibutyryl-cyclic AMP, a cAMP analogue, to mature cultured astroblastoma cells to differentiated astrocytes. Hertz, Hertz, Fosmark, and Schousboe (1989) modified this drug-induced morphological alteration technique to prepare primary astrocyte cultures that were biochemically and biophysically similar to their in vivo counterparts. Lately, the dynamics of cAMP signaling by βARs and its effect on astrocytic morphological changes were studied by Vardjan, Kreft, and Zorec (2014). Using a FRET-based Epac cAMP biosensor they showed that upon βAR activation, the astrocytes induce a 5–10% decrease in cell cross-sectional area and a 30–40% increase in cell perimeter, which were due to the activation of ACs and thereby elevation of cAMP levels in these cells. In 2016, Paco, Hummel, Pla, Sumoy, and Aguado (2016) concluded from a genome-wide transcriptional study that cAMP signaling causes the global transcriptome of astrocytes to become more differentiated. But not just astrocytes are known to undergo morphological changes induced by cAMP signaling. Nicol et al. (2007) showed that pulsatile photo-induced release of caged cAMP by AC1 in growth cones controls axonal guidance. Also, intracellular rises in cAMP levels was recently shown to inhibit astrocytic swelling upon trauma and hypotonicity (Vardjan et al., 2016). This highlights the involvement of cAMP in regulating astrocytic morphology which deserves to be further explored.

6 | ROLE OF ASTROCYTE cAMP SIGNALING IN BRAIN PATHOLOGY

Several recent reviews discuss the involvement of astrocytes in brain disease (e.g., Nedergaard, Rodriguez, & Verkhratsky, 2010; Pekny et al., 2016; Verkhratsky et al., 2012). For reasons that need not be repeated, a large part of the literature on the involvement of signaling in astrocytes in pathology revolves around Ca^{2+} homeostasis whereas literature on the involvement of cAMP signaling is scarce and mostly focused on the (mal)function of Gs/Gi-coupled GPCRs. Here, we will limit ourselves to discuss two cases that we think are illustrative of the putative involvement of compartmentalized astrocytic cAMP signaling and Ca^{2+}/cAMP signaling crosstalk in brain diseases.

6.1 | Gs-linked signaling in Alzheimer's disease and memory formation

A recent report by Orr et al. (2015) showed that the Gs-coupled adenosine receptor A_{2A} is expressed at higher levels in hippocampal astrocytes in brains from Alzheimer's disease patients, and that genetic ablation of the astrocytic A_{2A} gene in mice enhanced long-term memory. Conversely, expression of the chemo-genetic Gs-coupled receptor Rs1 in astrocytes and selective activation by GR-125487 reduced long-term memory but did not affect the initial learning (Figure 5). A potential link to glycogen metabolism in astrocytes was not discussed by the authors. This could be relevant since (as discussed above) glycogen metabolism is regulated by cAMP signaling and linked to long-term retention of memories (Alberini et al., 2017; Suzuki et al., 2011). There are, however, no studies on the potential of A_{2A}-Gs-mediated cAMP signals to affect glycogen metabolism so any link

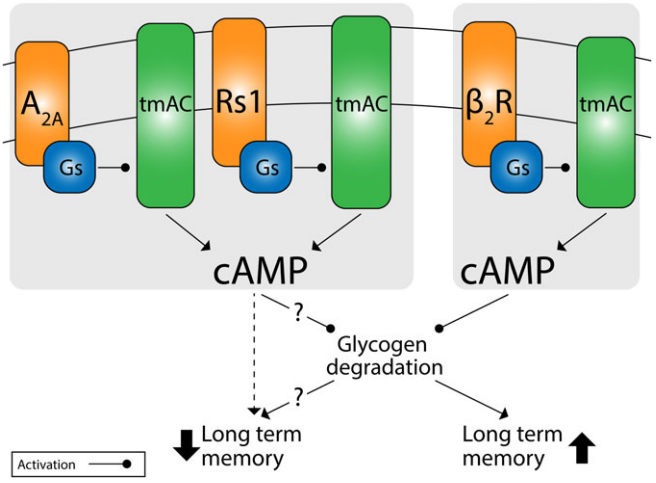

FIGURE 5 cAMP-mediated regulation of memory formation in astrocytes. Increased Gs-coupled adenosine receptor A_{2A} expression in hippocampal astrocytes may perturb long-term memory. Gs-coupled receptor Rs1 activation reduces long-term memory but does not affect the initial learning. Both effects are likely mediated through the activation of transmembrane bound adenylyl cyclases (tmACs) activation by Gs with subsequent cAMP formation, but whether glycogen metabolism is implicated remains elusive, as indicated by question marks. Conversely, β_2_receptor activation (β_2_R), through cAMP, leads to glycogen degradation, and enhanced long term memory formation. This example highlights a key feature of cAMP signaling, as cAMP might have different, or even opposite, downstream effects in cells depending on the compartmentalization of the signals

remains speculative at this point. Since glycogen metabolism seem to be required for long-term retention, but not for the initial learning (Suzuki et al., 2011), the enhanced long-term retention of memories caused by deletion of astrocyte A_{2A} receptors (Orr et al., 2015) suggests that the A_{2A}-mediated cAMP signal does not blend with the cAMP signal involved in regulating glycogen metabolism. Likewise, the effect of Rs1-mediated cAMP signals on glycogen metabolism has not been studied in relevant cells, but since activation of Rs1 expressed in astrocytes suppresses long-term retention of memories (Orr et al., 2015) it seems unlikely that the Rs1 signal interacts with the signals regulating glycogen. This also touches on the more general caveat of employing designer receptors exclusively activated by designer drugs (DREADDs) like Rs1 (a mutated serotonin receptor) to study physiological outcomes; with the knowledge we have now on the spatial compartmentalization of cAMP signals (and indeed all signaling pathways) we need a palette of DREADDs with well-defined intracellular signaling pathways and downstream signaling targets if we wish to employ these to study physiology and pathology. In the study by Orr et al., introducing Rs1 into animals produces the opposite effects of ablation of A_{2A} receptors but is it via the same signaling pathways and downstream effectors? The discussions in this review suggest that it is unlikely to be completely overlapping. Future studies should address whether cAMP signals from A_{2A} receptor activation can spill into the pathways regulating glycogen metabolism. Also, investigating the potential of Rs1-mediated signals to regulate glycogen metabolism in astrocytes could be useful.

6.2 | Potential interplay between astrocytic Ca^{2+} and cAMP signaling in epilepsy

Astrocytes seem to play a role in driving epileptic seizures although the role played by astrocytes in the development of epilepsy is less clear (e.g., reviews by Binder & Steinhauser, 2006; Verkhratsky et al., 2012). As reviewed by Nedergaard et al. (2010), astrocytic Ca^{2+} signaling has been shown to be aberrant in epileptic tissue typically showing an "enhanced" Ca^{2+} signaling phenotype which is also suggested by more recent reports that point to enhanced and longer-lasting spontaneous and induced Ca^{2+} transients in astrocytes from epileptic animal models (Alvarez-Ferradas et al., 2015; Szokol et al., 2015). Interestingly, astrocytic glycogen metabolism could be involved in the pathology of epilepsy since glycogen metabolism is intricately involved in the intermediary metabolism of astrocytes and to some extent fuels K^+ clearance following neuronal firing (DiNuzzo, Mangia, Maraviglia, & Giove, 2014; DiNuzzo, Mangia, Maraviglia, & Giove, 2015; Obel et al., 2012). Since we know that astrocytes express Ca^{2+}-activated isoforms of tmACs (Holtman et al., 2015) and that Ca^{2+}-mediated cAMP signals in astrocytes induce glycogen breakdown (Müller et al., 2014), it is tempting to speculate a role for this type of aberrant Ca^{2+}/cAMP signaling-metabolism coupling in astrocytes in the epileptic focus. Finally, this again highlights the need to always investigate the effects of observed changes in Ca^{2+} homeostasis on the concurrent cAMP signaling events.

7 | POTENTIAL NOVEL DRUG TARGETS

As discussed above, glycogen metabolism represents an example of a signaling-driven process that seems important for a number of processes, including Ca^{2+} homeostasis and formation of new memories. It is also becoming evident that cerebral glycogen metabolism is affected in a range of diseases including but not limited to type 2 diabetes, Alzheimer's disease, and epilepsy (DiNuzzo et al., 2015; Gibbs, 2015a; Sickmann & Waagepetersen, 2015). Thus, cerebral glycogen metabolism seems an attractive drug target in a range of conditions if an elevated glycogen metabolism is shown to be disease-modifying. If that is the case, then how may one go about targeting glycogen metabolism? Targeting the enzymes degrading glycogen seems like a challenge since hepatic and muscular glycogen metabolism would be affected causing side effects such as elevated blood glucose levels. A more feasible approach to target glycogen degradation (or any other cAMP downstream effect) could be to interfere with the specific cAMP signal. However, directly targeting the involved AC isoform(s) carries the risk of adverse effects due to the wide expression of ACs in different cell types. A more sensible approach might be targeting specific AKAPs or PDEs, since more than 50 AKAPs and almost 100 isoforms of PDE are known, and several of these are expressed in astrocytes (Table 1; Horvat & Vardjan, 2019). This could provide the possibility for targeting a specific cAMP signal selectively (Brescia & Zaccolo, 2016; Dessauer, 2009; Kokkonen & Kass, 2017; Kritzer, Li, Dodge-Kafka, & Kapiloff, 2012), although the same isoform of AKAP or PDE may be expressed in multiple cell types. Another approach

might be targeting protein–protein interactions (PPIs) in defined tmAC signaling hubs. This could be done by developing inhibitors of key PPIs in a given tmAC signaling hub, dislocating some downstream effector of cAMP from its substrate or maybe even its source of cAMP. This could turn out to be an even more selective approach but would require the identification and characterization of such complexes. At present, no such complexes have been studied in astrocytes.

8 | FUTURE DIRECTIONS AND CONCLUDING REMARKS

The tools are available for studying cAMP dynamics in vivo but before we embark on an in vivo cAMP imaging frenzy, it is important to sort out the underlying biochemistry in reduced model systems such as cell cultures, tissue slices, or tissue cultures. We need to understand the complex spatial and temporal dynamics of cAMP signals induced by different cues, and exactly how this interrelates with Ca^{2+}. To do this, new approaches need to be employed as commonly used pharmacological tools to manipulate cAMP cascades fall short in being able distinguish the intricacies of signaling compartmentalization. Such approaches should include a greater focus on investigating the functional importance of specific PPIs in signaling hubs, as these cannot be ignored given the current knowledge of cAMP signaling (Dema, Perets, Schulz, Deak, & Klussmann, 2015). Now is a great time for an expansion into astrocytic cAMP signaling research, as we believe the tools and the putative significance of such research have never been greater than they are now.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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