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Regulatory mechanisms for glycogenolysis and K^+ uptake in brain astrocytes

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Abstract

Recent advances in brain energy metabolism support the notion that glycogen in astrocytes is necessary for the clearance of neuronally-released K^+ from the extracellular space. However, how the multiple metabolic pathways involved in K^+-induced increase in glycogen turnover are regulated is only partly understood. Here we summarize the current knowledge about the mechanisms that control glycogen metabolism during enhanced K^+ uptake. We also describe the action of the ubiquitous Na^+/K^+ ATPase for both ion transport and intracellular signaling cascades, and emphasize its importance in understanding the complex relation between glycogenolysis and K^+ uptake.

Keywords

glycogen; glycogen phosphorylase; potassium; astrocytes; Na^+/K^+ ATPase

Introduction

In the brain, glycogen and glycogen phosphorylase activity are both confined to astrocytes (Pfeiffer-Guglielmi et al., 2003). Although astrocytes are not excitable cells, they are critically involved in the uptake of the excess K^+ released in the extracellular space by neurons during action and synaptic potentials (Hertz et al., 2007). These astrocytic competences, namely glycogenolysis and K^+ uptake, have been recently shown to be functionally linked. In cultured and tissue slice astrocytes, glycogen was found to fuel specifically K^+ uptake (Choi et al., 2012; Xu et al., 2013). Importantly, the astrocytic uptake of K^+ was abolished by inhibiting glycogenolysis using the glycogen phosphorylase inhibitor 1,4-dideoxy-1,4-imino-d-arabinitol (DAB) (Xu et al., 2013). Here we describe the regulatory mechanisms that are expected to couple K^+ uptake with glycogen mobilization in

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brain astrocytes (schematically summarized in Figure 1). The elucidation of the specific contribution of glycogen to cerebral energy demand under normal conditions (i.e. conditions not associated with hyper- or hypoglycaemia) is important for the characterization of the functional partnership between neurons and astrocytes, a cell-to-cell cooperation which forms the basis of the coupling between neuronal activation and metabolism (DiNuzzo et al., 2010; Mangia et al., 2009). In the choice of the topics we adopted an inclusive criterion by privileging the incorporation of all potentially relevant pathways even when the evidence for their involvement in K^+-induced glycogenolysis is lacking or poorly documented. Although this gives a speculative character to the present review, it helps systematization of the mosaic of findings that would remain otherwise unconnected.

Glycogenolysis is regulated by phosphorylation and allosteric control during enhanced K^+ uptake

Glycogen mobilization by glycogen phosphorylase (GP) is under phosphorylation as well as allosteric control mechanisms (Roach, 2002). Regulation by phosphorylation (Figure 1, pathways 2 and 3) involves the activation of phosphorylase kinase (PhK), which phosphorylates GP causing the transition from the normally inactive GPb (but see below) to the active GPa configuration of the enzyme. PhK contains four Ca^{2+} binding sites that normally inhibit the phosphotransferase activity, which becomes disinhibited when intracellular Ca^{2+} concentration increases (reviewed by Brushia and Walsh, 1999). Elevations in Ca^{2+} level during enhanced K^+ uptake can be the result of intracellular signaling cascades initiated by a transducer protein and mediated by phospholipase C (PLC) and inositol trisphosphate receptor (IP3R) (see for example Xu et al., 2013), but can also be elicited by activity of Na^+/Ca^{2+} exchanger (NCX) proteins and/or voltage-gated Ca^{2+} L-channels (LCC) on plasma membrane (Subbarao et al., 1995). Increase in K^+ uptake also induces alkaline shift in intracellular pH due to increased bicarbonate flux thorugh the Na^+/HCO_3^- cotransporter (NBC) (Brookes and Turner, 1994). The rise in HCO_3^- level results in the stimulation of HCO_3^--activated soluble adenylate cyclase (sAC) (Choi et al., 2012), which converts ATP to cAMP. Subsequent binding of cAMP to cAMP-dependent protein kinase A (PKA) leads to direct phosphorylation of PhK. Therefore, PhK itself is regulated both by covalent modifications and allosteric mechanisms.

The GPb form of the phosphorylase is not always inactive but can be activated allosterically (Figure 1, pathway 1) by AMP (Guenard et al., 1977). AMP is produced by adenylate kinase (AK), which amplifies small decreases in ATP concentration after increased cellular energy demand due to K^+ uptake (Hardie et al., 2011). Binding of AMP to GPb triggers conformational change of the enzyme from the tense (T) to the relaxed (R) state. The latter form has similar catalytic properties of the phosphorylated GPa enzyme. AMP can also stimulate the AMP-activated protein kinase (AMPK) both allosterically and by inhibiting dephosphorylation. AMPK accommodates a glycogen-binding domain (GBD) that may favor net glycogenolysis (e.g., by stopping glycogen synthesis) upon AMPK activation (Longnus et al., 2003; Polekhina et al., 2003). Interestingly, glycogen in turn regulates AMPK acting as an allosteric inhibitor of the kinase activity (McBride et al., 2009).

Contrary to GP, glycogen synthase (GS) is active in its non-phosphorylated GSa form and inactive when phosphorylated to GSb form (Roach, 2002). Reciprocal regulation of GS and GP by covalent phosphorylation does not however translate in mutually exclusive synthesis and degradation of brain glycogen. Indeed, simultaneously active GS and GP concur to produce the steady-state turnover, which in the human brain is about 0.16 $\mu mol \cdot g^{-1} \cdot h^{-1}$ (Oz et al., 2007). The fact that the rate of glycogen turnover at steady-state is not zero is probably due to the presence of allosteric effectors. For example, GS is allosterically activated by glucose 6-phosphate even when the enzyme is phosphorylated (see Roach et al., 2012). Furthermore, although at tissue/pool level the rate of synthesis must equal that of degradation, individual glycogen molecules can be found in different states of synthesis and degradation, such that locally the rate of synthesis and degradation are unmatched (DiNuzzo, 2013).

Astrocytic K^+ uptake occurs via Na^+/K^+-ATPase (NKA) and $Na^+/K^+/2Cl^-$ cotransporter (NKCC)

Uptake of excess extracellular K^+ by astrocytes is favored by the lower affinity of astrocytic NKA for K^+ relative to neuronal NKA, which is due to differences in the composition of the enzyme with respect to the catalytic α subunits (Crambert et al., 2000; Newman, 1995; Ransom et al., 2000). In particular, the neuron-specific α3 subunit makes the neuronal NKA already saturated for K^+ at basal extracellular K^+ levels (Munzer et al., 1994). It is likely that the difference in K^+ affinity at the extracellular K^+-sensitive site is determined by protein-protein interactions between NKA and a family of small membrane proteins regulating NKA activity named FXYD (Crambert and Geering, 2003). Among these, FXYD7 is exclusively expressed in the brain and decreases the apparent affinity for extracellular K^+ (Beguin et al., 2002). Notably, FXYD7 seems to associate with α1 but not α2 or α3 subunits of NKA (Beguin et al., 2002). The low affinity of the astrocytic NKA isozyme for K^+ at its extracellular K^+-binding site compared with the neuronal enzyme (Grisar et al., 1979; Hajek et al., 1996) indicates that FXYD7 binds to astrocytic but not neuronal NKA. Accordingly, the expression of NKA subunits is not uniform in different cellular compartments. Dendrites and astrocytes are enriched in α1 and α2 while α3 appears to be specific for axons and presynaptic terminals (Brines and Robbins, 1993; McGrail et al., 1991; Shibayama et al., 1993). This also suggests that the discrepancy between astrocytic and neuronal K^+ affinity is larger for astrocytes ensheating axons and much lower for astrocytes ensheating dendrites, in agreement with a specific role for astrocytic K^+ uptake during presynaptic activity, as previously suggested (DiNuzzo et al., 2012; DiNuzzo et al., 2011). Unfortunately, it is presently unknown whether NKA/FXYD7 complex undergoes some kind of regulation (e.g. phosphorylation, like other members of the FXYD family) during physiological brain activity. NKA α1 and α2 subunits are indeed regulated via phosphorylation by PKA and protein kinase C (PKC), both seemingly producing inhibition of ion transport activity (Cheng et al., 1997).

Increases in extracellular K^+ (e.g., from 3 mM up to 10 mM) stimulate NKA at its extracellular K^+-binding site. The low affinity of astrocytic NKA for extracellular K^+ results in stimulation of NKA-mediated K^+ uptake, which requires extrusion of intracellular Na^+.

At this stage, energy metabolism is stimulated by the ATP hydrolysis due to action of NKA. Higher values of excess K^+ in the extracellular space (12 mM or above) cause activation of NKCC1, which is highly expressed by adult astrocytes (Yan et al., 2001). The activation of NKCC1 ensures availability of intracellular Na^+ for NKA (Figure 1, NKA/NKCC/AE pathway). Thus, the concerted action of NKA and NKCC1 in astrocytes at high K^+ underlies a transmembrane Na^+ cycle and accumulation of K^+ (Walz, 1992). It should be noted that Na^+ enters astrocytes also via the procaine-inhibited Na^+ channel (Nax), which opens in response to increases in extracellular Na^+ (Figure 1, NKA/Nax pathway). The finding that inhibition of Nax channels by amiloride prevents K^+ uptake in cultured astrocytes (Xu et al., 2013) suggests that Nax might support the above-mentioned Na^+ cycle even before NKCC1 is activated. Blockade of the Nax-mediated return of Na^+ to the cell interior would increase the extracellular concentration of the ion, which is known to inhibit the external K^+-stimulated site of NKA (Skou, 1957, 2004). This argument is supported by the observation that ion transport activity of NKA is strongly inhibited by external Na^+ when the enzyme is associated with FXYD7 (Brines and Robbins, 1993; Geering, 2005). The effect of increased extracellular Na^+ in culture is likely to be absent in vivo, where Na^+ transiently decreases in the extracellular space because of the massive influx of the ion into neurons. The regulation of NKCC1-mediated ion transport via phosphorylation (e.g., by PKA) and its dependence on osmotic conditions are presently uncertain (Gosmanov and Thomason, 2003; Reynolds et al., 2007; Wong et al., 2001).

Role of $Na^+/HCO3^-$ cotransporter (NBC) in mediating K^+-induced glycogenolysis

Another route for Na^+ intake in astrocytes for the support of NKA action is the electrogenic NBC (Figure 1, NKA/NBC pathway). Recent studies reported that K^+ produces a substantial up-regulation of bicarbonate transport inside astrocytes via NBC (Choi et al., 2012; Ruminot et al., 2011). These experiments confirmed previous observations supporting net HCO_3^- uptake due to depolarization induced by increased extracellular K^+ (Brookes and Turner, 1994; Chesler and Kaila, 1992; Ransom, 1992). In this respect, a substantial contribution to HCO_3^- uptake can be due to the 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS)-inhibited, Na^+-driven anion exchanger (AE). AE might increase HCO_3^- uptake in exchange with intracellular Cl^-, which is substantially taken up by astrocytes along with K^+ via NKCC1. Extracellular HCO_3^- can be produced by the activity of surface carbonic anhydrase (CA) IV, which is the predominant enzyme isoform in astrocytes (Svichar et al., 2006). Interestingly, the rise in intracellular HCO_3^- was found to elicit stimulation of soluble adenylate cyclase (sAC) and production of cAMP as well as subsequent glycogen breakdown, which was inhibited by the sAC-selective blocker 2-hydroxyestradiol (Choi et al., 2012). Thus, the involvement of bicarbonate-induced glycogenolysis adds to the pathways leading to activation of PKA and PhK/GP phosphorylation cascade.

Involvement of NKA/Src/EGFR and Ras/Raf/MEK/ERK signaling cascades in astrocytic K^+ uptake

An important feature of NKA is its recently appreciated role as signal transducer for cardiotonic steroids (CTS) such as ouabain (Haas et al., 2000). Exposure of NKA to CTS, besides inhibiting ion transport, also activates intracellular signaling cascades. In particular, CTS result in release of the kinase domain of the nonreceptor tyrosine kinase Src from the normally inactive NKA/Src complex and Src activation. The latter triggers the release of an epidermal growth factor receptor (EGFR), which in turn mediates the activation of the Ras/Raf/MEK/ERK pathway (hereafter named ERK pathway for simplicity) as well as intracellular Ca^{2+} signaling and protein kinases (for a comprehensive review, see Schoner and Scheiner-Bobis, 2007).

It should be realized that the activation of the Src and ERK pathways identify the effects of CTS, which was proposed as an important physiological mechanism due to the presence of endogenous CTS-like compounds in the brain (Rosen et al., 2006), but was not otherwise connected to normal ion homeostasis. The recent study by Xu and colleagues on cultured astrocytes reported the involvement of these cascades during K^+-induced glycogenolysis (Xu et al., 2013). This finding is consistent with the fact that the same signaling pathways activated by CTS are also activated by inhibition of NKA independent of CTS. For example, inhibiting NKA by lowering extracellular K^+ promotes a rapid and large increase in ERK phosphorylation (Plourde and Soltoff, 2006). This suggests that Src activation is caused by down-regulation of NKA activity. In particular, Src might become activated because of the reduction of NKA activity after phosphorylation by PKA and PKC (see ‘Astrocytic K^+ uptake via Na^+/K^+-ATPase (NKA) and $Na^+/K^+/2Cl^-$ cotransporter (NKCC)’ section). In cultured astrocytes, both the exposure to 30 nM ouabain as well as addition of 5 mM or 10 mM K^+ cause increases in ERK phosphorylation, indicating the participation of the ERK pathway initiated by the NKA/Src/EGFR complex (Xu et al., 2013) (Figure 1, pathway 4). Given the above-mentioned considerations, this finding raises the question of why the ERK pathway is activated also by increased extracellular K^+. Therefore, it is likely that the pathway leading to Src activation is not only due to NKA inhibition, as evidenced by the fact that in multiple cell types Src is phosphorylated directly by PKA (Baker et al., 2006; Obara et al., 2004). In turn, PKA-mediated phosphorylation of several target proteins, including LCC, is inhibited by Src (Bogdelis et al., 2011). These results indicate that the interaction between PKA and Src has the potential to initiate ERK pathway and at the same time terminate the glycogenolytic effect. Notably, inhibition of glycogenolysis by DAB reduces ERK phoshorylation by nearly 40% at 10 mM extracellular K^+ (the reduction is even lower at 5 mM extracellular K^+), while it completely abolishes the astrocytic K^+ uptake (Xu et al., 2013). This finding suggests that neither K^+ uptake nor glycogenolysis are required for the activation of ERK pathway. Whether ERK phosphorylation is stimulated by other routes secondary to NKA/Src signaling and PKA activation during increased extracellular K^+ remains to be established.

Overall, while the involvement of glycogen in astrocytic K^+ uptake is established, the relation of cause-effect between glycogenolysis and the intracellular signaling cascades

requires further research. This includes direct monitoring of the effect of ERK phosphorylation on intracellular K^+ accumulation, which is especially important because there is evidence that activation of ERK pathway results in glycogen synthesis not degradation. Specifically, in human skeletal muscle (Kotova et al., 2006) as well as in human NT2 cell lines (Fridman et al., 2012), CTS have been found to elicit either accumulation or redistribution of glycogen granules, a mechanism mediated by Src and ERK pathways and involving inhibition of glycogen synthase kinase 3 (GSK3). GSK3 phosphorylates glycogen synthase (GS) converting it from the active GSa to the inactive GSb form of the enzyme, thus blockade of GSK3 relieves the inactivating mechanism of glycogen synthesis. It is thus possible that the pathway leading to ERK phosphorylation is the result not the cause of K^+ uptake and glycogen utilization, which might be useful to replenish the glycogen pool (Figure 1, pathway 4). It is noted that another potential mechanism for inhibition of GSK3 and stimulation of glycogen synthesis is the activation of NKA-bound phosphatidylinositide 3-kinase (PI3K) and the resulting protein kinase B (PKB/Akt) cascade (Schoner and Scheiner-Bobis, 2007).

Possible recurrent signaling between NKA, glycogen and Ca^{2+} signaling during enhanced K^+ uptake

Inhibition of inositol trisphosphate receptors (IP3Rs) by Xestospongine, which prevents the increase in intracellular Ca^{2+} levels, has been found to produce similar effects on K^+ uptake of inhibiting glycogenolysis with DAB (Xu et al., 2013). This finding would support a key role of allosteric stimulation of PhK by Ca^{2+} in mediating the K^+-induced glycogenolysis. Nonetheless, it has been found that also DAB suppresses significantly, though not completely, the rise in Ca^{2+}, suggesting that at least part of the Ca^{2+} increase is a consequence not a cause of glycogenolysis. The fact that K^+ uptake is abolished by preventing the IP3R-mediated increase in intracellular Ca^{2+} can be explained by the formation of a signaling microdomain between NKA and IP3Rs (Miyakawa-Naito et al., 2003). The latter interpretation is consistent with the observation that ouabain stimulates IP3Rs and Ca^{2+} signaling via a protein-protein interaction without the involvement of PLC (Aizman and Aperia, 2003) (Figure 1, pathway 3). It is likely that this process is dependent on Src kinase and ERK pathway, as the inhibition of Src and ERK blocks the ouabain-induced increase in intracellular Ca^{2+} (Tian et al., 2001). Although these data come from sparse studies on different cell cultures, they are in agreement with the hypothesis that inhibition of one element of the NKA signalosome, such as IP3Rs in this case, may interfere with normal NKA activity. At the moment and without invoking other regulatory mechanisms, a plausible candidate for this interference of astrocytic K^+ uptake is FXYD7. Detachment of FXYD7 from NKA would increase the enzyme affinity for extracellular K^+ and rapidly saturate the ion transport rate, thereby suppressing the subsequent K^+ effect.

Other experiments could not report any increase in astrocytic Ca^{2+} level after increase in extracellular K^+ in the range 5–10 mM (Choi et al., 2012; Duffy and MacVicar, 1994). This discrepancy is difficult to explain without invoking issues related to different cell or tissue preparations (see Hertz and Code, 1993; see also discussion in Xu et al., 2013). Previous studies investigating the intracellular messengers for K^+-induced glycogenolysis in the brain

showed that activation of GP occurs by a cAMP-independent and Ca^{2+}-dependent mechanism (Ververken et al., 1982). The role of Ca^{2+} ion in stimulating glycogen breakdown after K^+ uptake in astrocytes was then repeatedly confirmed (Hof et al., 1988; Subbarao et al., 1995). More recently, the dependency of astroytic K^+ uptake on Ca^{2+} was supported in cortical (Wang et al., 2012a) and cerebellar (Wang et al., 2012b) astrocytes. These latter experiments indeed showed that increases in cytosolic Ca^{2+} mediate the activation of NKA in a PKA-dependent manner. Thus, the above-mentioned findings that K^+ uptake may be Ca^{2+}-independent are even more surprising considering that PKA, which was identified as the primary mechanism in mediating the observed glycogenolytic response to K^+ (Choi et al., 2012), is known to activate LCC by phosphorylation and prevent their inactivation (see, for example Hell, 2010; Meuth et al., 2002) (Figure 1, pathway 3). It is not known whether NCX is also a target of PKA in astrocytes, but NCX stimulation by PKA in neurons was found to be comparatively much higher (He et al., 1998).

Failure of glucose to support astrocytic K^+ uptake after inhibition of glycogenolysis

One of the most surprising outcome about K^+-induced glycogen utilization is that the suppression of astrocytic K^+ uptake after inhibition of glycogenolysis could not be supported by glucose (Xu et al., 2013). This suggests that, like for Ca^{2+} signaling, some chemical signal moves from glycogen to NKA and not exclusively the other way around. In other words, the role of glycogenolysis appears to be not only that of providing ATP to fuel NKA. Quite oppositely, the results indicate that a Ca^{2+}-dependent, possibly bidirectional signaling between glycogen and NKA is necessary for NKA to be fully activated. Figuring out what kind of signal could proceed from activated glycogenolysis to NKA cannot be even tentatively approached with current knowledge. It is tempting to speculate that a role may be played by the inorganic-phosphate-mediated association between glycogen and K^+ (Fenn, 1939; Poppen et al., 1953). This association might be altered by the combined action of NKA, which causes the rise in the level of inorganic phosphate due to enhanced ATP hydrolysis, and glycogenolysis, which causes the exposure of more glucosyl residues due to debranching of the glycogen molecule.

Glucose was able to fuel the K^+ uptake only when intracellular Na^+ was increased by stimulating Nax channels through increase in extracellular Na^+, which was achieved by the addition of either sodium pyruvate or the ionophore monensin. This finding is somewhat puzzling, but suggests that the preference of the NKA for glycogenolytic- or glycolytic-derived energy may depend on whether NKA is activated on the extracellular K^+-binding site or the intracellular Na^+-binding site, respectively.

Relative importance of AMP, HCO_3^-/cAMP/PKA/PhK and Ca2+/PhK routes in K+-induced glycogenolysis

Since glycogen phosphorylase in brain astrocytes is regulated through both phosphorylation by PhK and allosteric activation by AMP, understanding the relative importance of these mechanisms should be given priority. For example, closer look at the effect of excess

extracellular K^+ on cAMP (Figure 2 in Choi et al., 2012) suggests that less than 20% increase in cAMP level at 10 mM K^+ would be far from what generally is needed to stimulate glycogenolysis. Furthermore, quantitative analysis of NBC function and its dependence on NKA-established gradients compared to K^+ uptake indicated that the rate of NBC activity normally is at least several times lower than NKA/NKCC activity in astrocytes (see discussion in Peng et al., 2012). Eventually, Ca^{2+}-activated PhK received the strongest support as the primary target enzyme for regulation of GP (see 'Possible recurrent signaling between NKA, glycogen and Ca^{2+} signaling' section). This would identify the mixed phosphorylation/allosteric activation route by increased Ca^{2+} concentration as the major factor during K^+-stimulated glycogenolysis, according to a similar effect acting in muscle (Ozawa, 2011). Nonetheless, as allosteric control is faster than enzyme phosphorylation cascades, GPb might be crucial in the first seconds of enhanced energy demand before covalent modification to the enzyme takes place (Walcott and Lehman, 2007). The very high sensitivity to AMP exhibited by brain GPa and GPb is clearly evidenced by their high AMP binding affinity (low K_m), which is substantially higher compared with muscle isoforms (Guenard et al., 1977; Lowry et al., 1967). On the other hand, the low affinity (high K_m) of brain GPa and GPb for glycogen implies poor enzyme activity at low AMP concentration regardless of phosphorylation states (see discussion in Crerar et al., 1995). The phosphorylation state of muscle GP has instead a large impact on enzyme activation (Lowry et al., 1967).

In quantitative terms, the relative contribution of phosphorylation versus allosteric control of glycogen phosphorylase remains to be elucidated. It should be kept in mind, however, that the activation of PKA by cAMP also stimulates the conversion of cAMP to AMP by soluble phosphodiesterase (PDE) IV, which exhibits high affinity for cAMP and is activated in response to phosphorylation by PKA (Madelian and La Vigne, 1996). This means that allosteric and phosphorylation activation mechanisms are inter-related. Activation of PhK was also reported to be mediated by autophosphorylation-dependent protein kinase in a cAMP- and Ca^{2+}-independent pathway (Yu and Yang, 1995). Finally, the spatiotemporal dependence of K^+-induced glycogenolysis should be taken into account when determining changes in GP activity produced by a specific pathway. However, this detailed characterization is experimentally challenging, and adds to the limitations represented by tissue or cell culture preparations.

Concluding remarks

The notion that glycogen in astrocytes is required for sequestration of excess extracellular K^+ after neuronal activity (Xu et al., 2013) represents a great advance in brain energy metabolism (Mangia et al., 2013). However, the control of this mechanism is only partly understood. The point arises from the very enzyme that degrade glycogen, which is subjected to regulatory mechanisms that pertain to apparently different aspects of cell metabolism, namely ATP turnover and intracellular signaling. Furthermore, NKA, the protein that mediates the relation between K^+ uptake and glycogen has recently underwent a substantial reconsideration, because it does not only hydrolyse ATP for transporting ions but it has a key role as signal transducer. The failure of glucose to replace glycogen in K^+ uptake is possibly due the complex signaling mechanisms between NKA and Src, IP3Rs,

FXYD7, and their targets including NKA itself. Important experimental challenges for future research will include the elucidation of how the regulatory mechanisms described in this paper shapes the cause-effect relationship between K^+ uptake and glycogenolysis in the brain.

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Abbreviations used

AE	anion exchanger
AMPK	AMP-activated protein kinase
CA	carbonic anhydrase
CTS	cardiotonic steroids
DAB	1,4-dideoxy-1,4-imino-d-arabinitol
DIDS	4,4′-diisothiocyanostilbene-2,2′-disulfonic acid
EGFR	epidermal growth factor receptor
ERK	extracellular-signal regulated kinase
GBD	glycogen binding domain
GP	glycogen phosphorylase
GS	glycogen synthase
GSK3	glycogen synthase kinase 3
IP3R	inositol trisphosphate receptor
LCC	L-type voltage-dependent Ca^{2+} channel
MEK	mitogen-activated protein and extracellular-signal regulated kinase
Nax	extracellular Na^+ level sensitive Na^+ channel
NBC	Na^+/HCO_3^- cotransporter
NCX	$Na^+/Ca2^+$ exchanger
NKA	Na^+/K^+ ATPase
NKCC	$Na^+/K^+/2Cl^-$ cotransporter
PDE	phosphodiesterase
PhK	phosphorylase kinase
PI3K	phosphatidylinositide 3-kinase
PKA	cAMP-dependent protein kinase A
PKB/Akt	protein kinase B

PKC	protein kinase C
PLC	phospholipase C
Raf	rapidly accelerated fibrosarcoma
Ras	rat sarcoma
sAC	soluble adenylate cyclase

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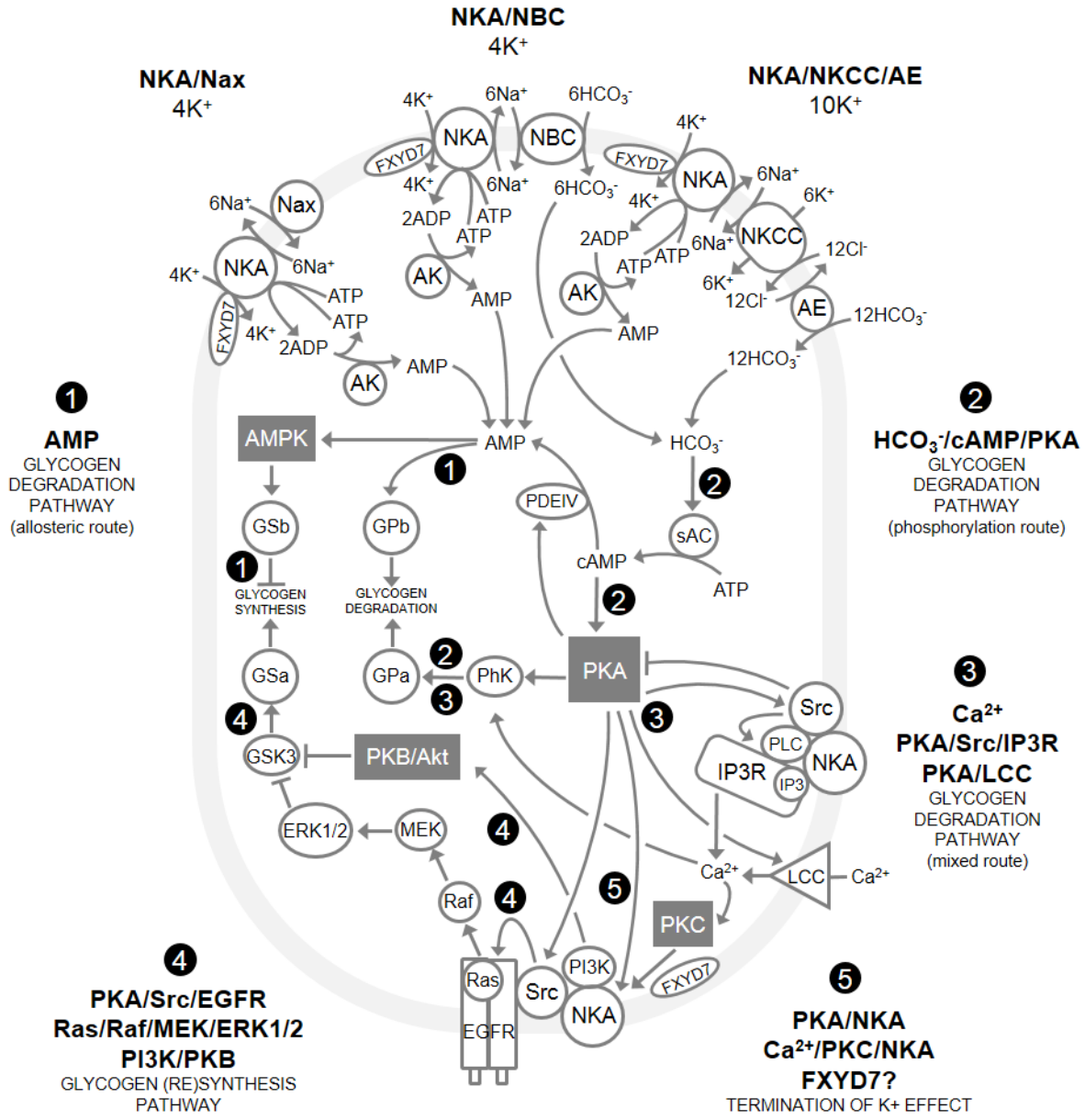

Figure 1. Metabolic and signaling pathways for K^+-induced glycogen degradation and resynthesis

Electrical activity of neurons results in the release of large amounts of K^+ by these cells into extracellular space. As the neuronal Na^+/K^+ ATPase (NKA) is already saturated for extracellular K^+, a substantial fraction of the excess extracellular K^+ is taken up by low affinity astrocytic NKA/FXYD7. NKA action hydrolyzes ATP to ADP and thus stimulates adenylate kinase (AK) to produce AMP. AMP allosterically activates glycogen phosphorylase (GP) and glycogen degradation. AMP also stimulates AMP-activated protein kinase (AMPK), which phosphorylates glycogen synthase (GS) thereby inhibiting glycogen synthesis, which favors net glycogenolysis. K^+ uptake by NKA is accompanied by Na^+ extrusion. Thus, intracellular Na^+ is necessary for NKA activity. Availability of Na^+ inside astrocytes is supported by extracellular Na^+ level sensitive Na^+ channel (Nax, top left),

sodium bicarbonate cotransporter (NBC, top middle) and $Na^+/K^+/2Cl^-$ cotransporter plus anion exchanger (NKCC/AE, top right). Note that the NKCC/AE is the most efficient system for extracellular K^+ clearance, as indicated by the net ion transport underlying the hydrolysis of 1 ATP to AMP plus 2 inorganic phosphates. Bicarbonate anion (HCO_3^-) is taken up because of the concerted action of NBC and possibly NKCC plus AE. HCO_3^- activates soluble adenylate cyclase (sAC) and downstream cyclic AMP (cAMP) production and cAMP-dependent protein kinase A (PKA) activation. PKA has several target proteins including glycogen phosphorylase kinase (PhK), which phosphorylates GP resulting in further glycogen degradation. Note that AMP is still being produced by the conversion of cAMP to AMP by the PKA-stimulated phosphodiesterase IV (PDEIV). PKA phoshorylates L-type Ca^{2+} channels (LCC) and perhaps $Na^+/Ca2^+$ exchanger (NCX) and NKCC leading to increase in Ca^{2+} influx. Cytosolic Ca^{2+} can also be liberated from intracellular stores through activation of inositol trisphosphate receptor (IP3R), which is part of the NKA signalosome. Ca^{2+} allosterically stimulates PhK, GP phosphorylation and glycogenolysis. NKA can be inhibited via phosphorylation by PKA and Ca^{2+}-activated protein kinase C (PKC), resulting in the stimulation of NKA-bound Src kinase. PKA directly phosphorylates Src, which initiates through an extracellular growth factor receptor (EGFR) the extracellular-signal regulated kinase (ERK) cascade. ERK phosphorylation inhibits glycogen synthase kinase 3 (GSK3) resulting in glycogen resynthesis. The same effect on GSK3 can be produced by the phosphatidylinositide 3-kinase/protein kinase B (PI3K/PKB) pathway. Finally, active Src in turn inhibits PKA and thus represents a primary candidate for terminating the K^+-induced glycogenolytic effect.