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Metabolic pathways and activity-dependent modulation of glutamate concentration in the human brain

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Abstract

Glutamate is one of the most versatile molecules present in the human brain, involved in protein synthesis, energy production, ammonia detoxification, and transport of reducing equivalents. Aside from these critical metabolic roles, glutamate plays a major part in brain function, being not only the most abundant excitatory neurotransmitter, but also the precursor for γ -aminobutyric acid (GABA), the predominant inhibitory neurotransmitter. Regulation of glutamate levels is pivotal for normal brain function, as abnormal extracellular concentration of glutamate can lead to impaired neurotransmission, neurodegeneration and even neuronal death. Understanding how the neuron-astrocyte functional and metabolic interactions modulate glutamate concentration during different activation status and under physiological and pathological conditions is a challenging task, and can only be tentatively estimated from current literature. In this paper, we focus on describing the various metabolic pathways which potentially affect glutamate concentration in the brain, and emphasize which ones are likely to produce the variations in glutamate concentration observed during enhanced neuronal activity in human studies.

Keywords

aspartate; glutamate; human brain; homeostasis; in vivo studies; malate-aspartate shuttle; neuron-astrocyte interactions; neurotransmission; neuronal stimulation

The excitatory action of cerebral glutamate and the importance of glutamate reuptake and conversion to glutamine were discovered in the 1950s and 1960s [reviewed by 1]. These findings were surprising, since the amino acid was known to have already enough ‘ordinary’ metabolic roles to be further selected by evolution as the major excitatory neurotransmitter in the nervous system of mammals [2]. In fact, aside from being one of the amino acids used to make proteins, glutamate is directly involved in critical metabolic functions such as energy production via exchange with the tricarboxylic acid (TCA) cycle intermediate α -ketoglutarate (AKG), and ammonia detoxification via the astrocytic conversion of glutamate to glutamine. Glutamate is also metabolically related to aspartate and is the precursor for γ -aminobutyric acid (GABA), the predominant inhibitory neurotransmitter. An interesting argument is that the selection pressure for related, readily inter-convertible compounds with opposite effects on cell membrane receptors (i.e. excitatory versus inhibitory) and potential (i.e. depolarization versus hyperpolarization) favored glutamate and its decarboxylated

derivative GABA [3,4]. The evolution might have exploited the link between glutamate and GABA that existed before neurotransmitters came into being. In particular, during failure of oxidative metabolism there is a decrease in glutamate and a concomitant increase in GABA due to reduction of glutamate synthesis as well as GABA breakdown, both oxygen-dependent processes. This was probably an efficient protecting mechanism in the brain to reduce neuronal activity and energy consumption, at least in some heterothermic, anoxia-tolerant species capable of sustained depression of brain metabolic rate [3].

Both neurons and astrocytes are endowed with a variety of glutamate receptors such as the ionotropic NMDA, AMPA/kainate and the metabotropic G protein-coupled receptors, which modulates both the short-term and long-term communications between cells during normal brain functioning. Furthermore, activation of glutamate receptors has profound consequences on intracellular signaling cascades as well as on ionic movements and associated energy supply and demand balance (e.g. neurovascular and neurometabolic coupling). Given the complex interplay of metabolic and functional mechanisms for cerebral glutamate homeostasis, disturbances in glutamate-mediated neurotransmission are implicated in virtually all neurologic and neuropsychiatric disorders. As reviewed in [5], a dysfunction of glutamate transporters leading to excitotoxicity has been implicated in the development of a variety of diseases, such as Alzheimer's disease [6,7], epilepsy [8], Huntington's disease [9,10], hepatic encephalopathy [11,12]. Recent lines of evidence recognize a critical role of glutamate also in major depressive disorders [13] as well as in schizophrenia [14] and impulsive behavior [15].

Whereas a detailed analysis of the involvement of glutamate in the pathogenesis and progression of different brain disorders falls beyond the scope of the present contribution, here we focus on describing which pathways are likely to produce the variations in glutamate concentration observed during paradigms of functional stimulation of the human brain. To this aim, we first summarize the mechanisms which are responsible for glutamate homeostasis in the human brain, we then refer to the functional and metabolic pathways which critically involve glutamate both in astrocytes and neurons, and we finally put those in the context of the experimental evidence regarding the dynamics of glutamate levels during functional studies of the human brain.

Homeostasis of cerebral glutamate

Glutamate is the most abundant excitatory molecule present in the human brain [16,17], even if, quite surprisingly, it took a long time to fully recognize it as a neurotransmitter [18]. Glutamate is predominantly distributed inside neuronal cytoplasm with a concentration of 10–15 mM [19], which allows fast packing into synaptic vesicles at the nerve terminals up to concentrations as high as 100 mM inside. The cytoplasmic concentration in astrocytes is lower, in the range 0.1–5 mM [19], which is likely due to the presence of glutamine synthetase (see below). Rapid removal of glutamate from the synaptic cleft by astrocytic high affinity glutamate transporters maintains the extracellular concentration of the neurotransmitter in the range of few μM [20]. Keeping glutamate mostly in the intracellular space is mandatory not only to avoid saturation of glutamate receptors in the postsynaptic neurons and thus ensure continuous neurotransmission, but also to avoid neurodegeneration or neuronal death by excitotoxicity. In fact, whereas glutamate is practically inactive inside cells, non-physiological exposure of neurons to extracellular glutamate can be highly toxic [1].

For proper neurotransmission, the refilling of the presynaptic neuronal glutamate pool is crucial. Quick removal of glutamate from the synaptic cleft, along with its anaplerotic replenishment during neurotransmission are all together supported by the glutamate-

glutamine cycle, which is strongly compartmentalized between neurons and astrocytes. In fact, the synthesis of glutamine from glutamate is exclusively glial [21], whereas the deamidation of glutamine to yield glutamate is primarily neuronal [22]. After release to the synaptic cleft, glutamate is thus taken up by astrocytes, where it is eventually returned to neurons as glutamine, which is an inactive molecule for glutamate receptors. In neurons, glutamine is finally converted back to glutamate by mitochondrial phosphate-activated glutaminase (PAG). The glutamate-glutamine cycle is therefore tightly linked to neurotransmission.

Glutamate removal is mainly achieved by the glutamate-glutamine cycle, but it is also accomplished by other mechanisms in the human brain, namely the direct neuronal glutamate uptake, the role of which is of functional importance during neurotransmission [23,1,24,25]. Finally glutamate can efflux to the blood at the level of the blood-brain-barrier, as supported by the presence of Glu transporters on the endothelial cells [26–29].

Glutamate metabolism in neurons and astrocytes

Glutamate is a central compound in cellular metabolism and its carbon skeleton is diverted to many diverse anabolic and catabolic pathways. This can occur only after it is sequestered from the TCA cycle at the level of AKG, implying that anaplerotic reactions are required to prevent depletion of TCA cycle intermediates. Anaplerosis of TCA cycle is normally not significantly accomplished by neurons. In fact, these cells lack pyruvate carboxylase (PC), which is thought to represent the primary anaplerotic pathway in the brain [30]. Moreover, based on the current literature, it is difficult to draw any quantitative conclusion about the significance of other anaplerotic pathways in neurons. Under physiological conditions cerebral phosphoenolpyruvate-carboxykinase (PEPCK) and malic enzyme (ME) work in the direction of pyruvate formation, not towards CO₂ fixation [31,32]. The remaining option for neuronal anaplerosis is conversion of propionyl-CoA to succinyl-CoA, which can be realized either via fatty acids oxidation or via metabolism of essential amino acids by involvement of branched-chain keto acid dehydrogenase complex (BCKDC). The first pathway is probably not feasible in neurons, since fatty acids appear to be low in the brain [33] and likely restricted to astrocytes [34]. Conversely, neurons [35] and possibly glial cells [36] contain BCKDC. Essential amino acids such as valine, leucine, and isoleucine are therefore quickly metabolized by the brain, and the presence of BCKDC in neurons indicates that some carbon from the corresponding keto acids (-ketoisovalerate, -ketoisocaproate, and -keto- -methylvalerate, respectively) can ultimately result in the production of acetyl-CoA and propionyl-CoA in these cells. This pathway has been found to have potential clinical relevance, as treatment with precursors of propionyl-CoA improved the neurological status of a newborn with PC deficiency (see [37] and references therein). Nonetheless, the contribution of neuronal anaplerosis to de novo glutamate synthesis in the brain under normal circumstances remains unknown, therefore we assume that most of brain glutamate is synthesized in PC-competent glial cells, namely astrocytes [38,39]. Astrocytes are additionally enriched in high affinity excitatory amino acids transporter (EAAT) proteins. Uptake of glutamate after synaptic as well as non-synaptic release identifies one of the essential functions of astrocytes, i.e. clearance of extracellular space from neurotransmitter molecules via the glutamate-glutamine cycle.

The fate of the amino acid within brain cells includes (i) new amino acid synthesis (e.g. conversion of glutamate to ¹-pyrroline-5-carboxylate is a critical step in the biosynthesis of proline, ornithine, arginine and citrulline), (ii) entry in the TCA cycle after deamination to AKG by glutamate dehydrogenase (GDH) or by transaminase (for example, aspartate aminotransferase, AAT, branched-chain aminotrasferases, BCATs, or alanine aminotransferase, ALAT) for oxidation and possibly gluconeogenesis predominantly in

astrocytes, (iii) transport across inner mitochondrial membrane through aspartate-glutamate carrier (Aralar/AGC1) for transamination by AAT within the malate-aspartate shuttle (MAS), (iv) conversion in GABAergic neurons to GABA via decarboxylation by glutamate decarboxylase (GAD), (v) condensation with cysteine by glutamate-cysteine ligase (GCL) for glutathione synthesis, and finally (vi) amidation to glutamine only in astrocytes by glutamine synthetase (GS). Understanding the relative contribution of these processes to the neuron-astrocyte functional and metabolic interactions, and how this is modulated by the activation status of the brain under physiological and pathological conditions is apparently a difficult task and can only be tentatively estimated from current literature.

All the above-mentioned pathways for glutamate metabolism, including the glutamate-glutamine cycle, interface between each other according to cellular requirements [40]. Yet, the effective stoichiometry and direction of transport and reactions is governed by cytosolic and mitochondrial compartmentation as well as specific expression of enzymes and transporters. Unfortunately, this is not without inconsistency when compared with the available knowledge. For example, the possible absence of the glutamate/hydroxyl carrier (GC) in brain mitochondria would require rigid coupling between AGC1 and mitochondrial AAT (mAAT) [41]. Indeed, to maintain mitochondrial aspartate level necessary for MAS activity, the entry of glutamate through AGC1 must be followed by transamination with oxalacetate by mAAT. Nonetheless, significant processing of glutamate via GDH in mitochondria, which does not regenerate aspartate, was often reported both in neurons and in astrocytes [42]. Resort to another NADH mitochondrial shuttle (e.g. glycerol-3-phosphate shuttle) during up-regulation of GDH activity would partly reconcile the above-mentioned discrepancy. Furthermore, the expression of GC may provide substrate for GDH independently of intramitochondrial aspartate [43]. Finally, the determination of GDH activity in human brain must take into account the expression of two distinct enzymes: GDH1 is expressed in all mammals, while GDH2 is specific to humans and other primates. Notably, GDH2 exhibits very different basal activity and allosteric regulation, which may increase glutamate flux through the oxidative deamination pathway during enhanced neurotransmission [44].

As an approximation, we can devise three different states of cerebral glutamate metabolism: (i) a steady-state in which glutamate recycling is coupled with MAS (that is, glutamate-glutamine cycle and MAS are stoichiometrically balanced, whatever the mixing between glutamate pools), (ii) a glutamate-synthesis state in which there is net glutamate production by astrocytes, and (iii) a glutamate-oxidation state in which there is net glutamate consumption by astrocytes, neurons or both (Figure 1). After glutamate is channeled to TCA cycle for oxidation, its carbons can exit the cycle in the form of a glycolytic intermediate. Neurons are enriched in mitochondrial malic enzyme (mME) and can thus convert malate to pyruvate (although pyruvate recycling is probably not very important in neurons), whereas both neurons and astrocytes can convert oxalacetate to phosphoenolpyruvate via the gluconeogenic enzyme PEPCK.

It can be hypothesized that the different states of glutamate metabolism depend on the level of exocytosis of the transmitter molecule (either via neurotransmission by neurons or gliotransmission by astrocytes). This is in agreement with the observation that the fraction of glutamate oxidation versus recycling within astrocytes is a function of the extracellular glutamate concentration [45]. In particular, amidation of glutamate to glutamine via GS accounts for most of the processing of glutamate at low concentration of the amino acid in the extracellular space. As the extracellular glutamate level rises, the fraction of glutamate oxidized for energy production increases substantially relative to that converted to glutamine [46–48]. The fact that the conversion of glutamate to glutamine is an important determinant in the fate of glutamate after uptake by astrocytes is consistent with the substantial increase

in the utilization of glutamate as energy substrate following inhibition of GS by methionine sulfoximine, which might eventually result in stimulation of gluconeogenesis and glycogenesis [49 and references therein]. Still, it remains to be established to what extent glutamate fuels its own reuptake at different levels of neurotransmission.

Changes in glutamate concentration during enhanced neuronal activity

A major body of work over the last couple of decades has long implied an approximately 1:1 relationship between estimates of the rate of the glutamine-glutamine cycle and measures of oxidative glucose consumption rates in the brain of animals under different level of anesthesia [50 and references therein], thus establishing a clear link between average metabolic needs of the brain tissue and neurotransmission events. Notably, net changes in brain glutamate level during enhanced neuronal activity might be or might be not a consequence of glutamate release and recycling per se.

We have previously reported a slight increase in glutamate concentration ([Glu]) during stimulation of human primary visual cortex [51], which was confirmed by others [52]. In these studies, changes induced by physiological activity in tissue level of glutamate were measured among other metabolites in the human brain. Interestingly, the observed $3\pm 1\%$ increase in [Glu] during stimulation as compared to following resting periods was accompanied by a concomitant $15\pm 6\%$ decrease in aspartate concentration ([Asp]). Opposite changes in [Glu] and [Asp] were also reported in rat brain after generalized sensory stimulation [53], suggesting an interpretation related to the metabolic, rather than the transmitter role of glutamate. In particular, those variations were proposed to reflect the rate-limiting step identified by AGC1 within the MAS [51]. It is possible that the effect on AGC1 is secondary to a change in the equilibrium position of the mAAT-catalyzed transamination reaction. Indeed, increased flux of pyruvate through pyruvate dehydrogenase leads to increased levels of acetyl coenzyme-A (acetyl-CoA), which condenses with oxalacetate (OAA) to yield citrate via the mitochondrial enzyme citrate synthase (CS). In particular, acetyl-CoA successfully competes for the OAA available inside mitochondria, thus removing one of the molecules required by the transamination reaction [54 and references therein]. Sequestration of OAA by CS moves chemical equilibrium of the reaction $(\text{GLU} + \text{OAA} \leftrightarrow \text{ASP} + \text{AKG})$ to the left in order to maintain the AAT equilibrium constant $(\text{ASP} \times \text{AKG})/(\text{GLU} \times \text{OAA})$. The activity of cAAT, which does not suffer from substrate depletion, in turn determines, as net effect, an accumulation of glutamate and a withdrawal of aspartate in the cytosol (Figure 2). In this case, the increase of tissue [Glu] observed in response to stimulation is clearly suggestive of an up-regulated oxidative metabolism under fully aerobic conditions, and is only indirectly related to neurotransmission.

It is possible that the observed changes in [Glu] and [Asp] primarily reflect the neuronal compartment because of the following: (i) neurons account for a larger volume fraction compared with astrocytes (50% and 20%, respectively); (ii) astrocytic Aralar/AGC1 represents only 7% of the total protein expression in the brain; (iii) astrocytes possess higher ability to replenish intramitochondrial OAA due to expression of PC.

A closer look at the results of the functional MRS study by Mangia and colleagues seems to indicate that absolute changes in [Glu] (~ 0.3 mol/g) slightly overcame those of [Asp] (~ 0.2 mol/g), although those data could not ascertain such mismatch with statistical significance, neither was this finding confirmed by more recent studies [52]. The reason for a putative discrepancy in the absolute values of concentration changes of Glu and Asp is unknown, but it may reside in the action of GDH and/or a transaminase inside mitochondria. Future research should address the specific cellular expression and activity of Aralar/AGC1 and

glutamate/hydroxyl carrier [55]. A small amount of de novo synthesis of neurotransmitter in astrocytes might as well be the reason for a subtle glutamate surplus relative to aspartate during physiological stimulations.

A complete mismatch of [Glu] and [Asp] was instead observed in a study of the metabolic correlates of paroxysmal electric activity, where a large increase in [Glu] of about 2 mol/g was found to occur in absence of a corresponding decrease of [Asp] during the first few minutes of stimulation in the epileptic focus of a patient suffering from fixation-off sensitivity [56]. Such mismatch suggests that the abnormal increase in glutamate level might originate from mechanisms other than MAS. Indeed, given its magnitude the increased [Glu] could not be accounted for by an impaired glutamate uptake by astrocytic glutamate transporters alone because of the relatively small extracellular volume, while suggesting deficient activity of glutamine synthetase, a de novo synthesis of glutamate, or both. However, inhibition of glutamine synthetase (which would allow glial accumulation of glutamate) is thought to augment oxidation of glutamate and glycogen synthesis [49]. Therefore such a large increase in [Glu] most likely involves new synthesis of the neurotransmitter (Figure 1B). A transient increase in glutamate, but not aspartate content was also reported in the brain of chicks immediately after learning [57]. The transient [Glu] increase related to pyruvate carboxylation in astrocytes can occur before glutamate is oxidized again in the glial TCA cycle, possibly after exchange with neurons via glutamate-glutamine cycling and neurotransmission [58]. Notably, in the study by [57], glycogen was reported to be preferred precursor of the newly synthesized glutamate, as also reviewed in [59], which is consistent with the hypothesis of a glycogenolysis stimulation induced by spiking activity [60], e.g. during the epileptiform seizure.

Conclusion

Several metabolic pathways which involve glutamate in the human brain are tightly connected to neuronal activity, and can potentially produce activity-dependent changes in glutamate content. Identifying which metabolic scenarios are likely to support changes in glutamate levels during stimulation is critical for characterizing the metabolic correlates of overall brain function. When increases of glutamate levels during stimulation are accompanied by matched decreases of aspartate levels, those changes in metabolite concentrations are consistent with a different equilibrium position of the transamination reaction catalyzed by mAAT and in turn of the AGC1 carrier. The resulting reduction in glutamate-aspartate exchange across mitochondria likely originates from sequestration of OAA by CS induced by increased pyruvate metabolism. Its occurrence during a rise in the overall MAS activity is in agreement with the up-regulation of non-oxidative metabolism observed in the stimulated brain. To the contrary, those changes in [Glu] which are mismatched relative to [Asp] decreases can possibly be accounted for by de novo synthesis of glutamate.

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Abbreviations

AKG	α -ketoglutarate
ALAT	alanine aminotransferase
Aralar/AGC1	aspartate-glutamate carrier
BCAT	branched-chain aminotrasferases
BCKDC	branched-chain keto acid dehydrogenase complex
cAAT	cytosolic aspartate amino-transferase
cME	cytosolic malic enzyme
CS	citrate synthase
DIC	dicarboxylate carrier
EAAT	excitatory amino acids transporter
GABA	γ -aminobutyric acid
GAD	glutamate decarboxylase
GC	glutamate/hydroxyl carrier
GCL	glutamate-cysteine ligase
GDH	glutamate dehydrogenase
GS	glutamine synthetase
mAAT	mitochondrial aspartate amino-transferase
MAS	malate-aspartate shuttle
MCT	monocarboxylate transporter
(m)ME	(mitochondrial) malic enzyme
OAA	oxalacetate
PAG	phosphate-activated glutaminase
PC	pyruvate carboxylase
PEPCK	phosphoenolpyruvate-carboxykinase
TCA	tricarboxylic acid.

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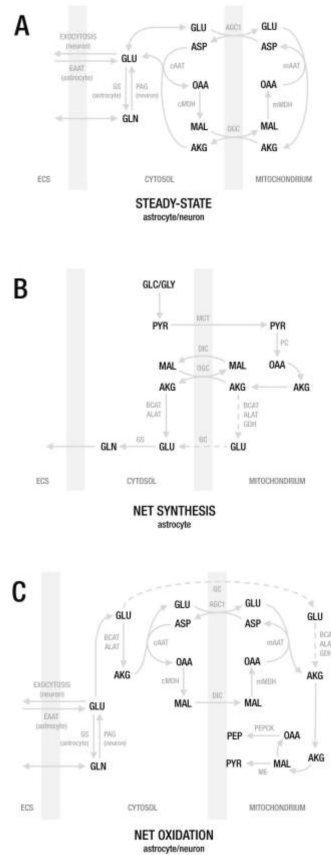


Figure 1. Metabolic pathways for cerebral glutamate recycling, net synthesis and net oxidation (A) Steady glutamate recycling. Glutamate cycling and MAS are stoichiometrically balanced. The two pools of intracellular glutamate (i.e. derived either from EAATs in astrocytes, PAG in neurons or cAAT in both cell types) might eventually undergo mixing. (B) Net glutamate synthesis. Pyruvate generated by glycolysis or glycogenolysis in astrocytes is transported inside mitochondria by monocarboxylate transporter (MCT). Pyruvate is then converted by PC to oxalacetate, whose carbons exit TCA cycle as AKG. Intramitochondrial AKG is exchanged with malate via oxoglutarate carrier (OGC). Subsequent countertransport of malate with phosphate through dicarboxylate carrier (DIC) ensures net AKG shuttle across mitochondria without net transfer of malate. Once in the cytosol, AKG is transaminated to glutamate by branched-chain aminotransferase (BCAT) or alanine aminotransferase (ALAT). Glutamate is aminated to glutamine by GS and exported to neurons. Dashed arrows show the alternative pathway requiring glutamate/hydroxyl carrier (GC). (C) Net glutamate oxidation. Cytosolic glutamate enters mitochondria via AGC1, though not directly, but after two transamination steps mediated by BCAT or ALAT and cAAT (the alternative pathway using GC is shown with dashed arrows). Carbons from intramitochondrially generated AKG exits TCA cycle as malate or oxalacetate, which are then converted to pyruvate. Transport of malate via dicarboxylate carrier (DIC) is required for net glutamate entry and exit to and from mitochondria (unless GC is operative, dashed arrows). Note that activity of mitochondrial GDH requires GC to balance stoichiometry.

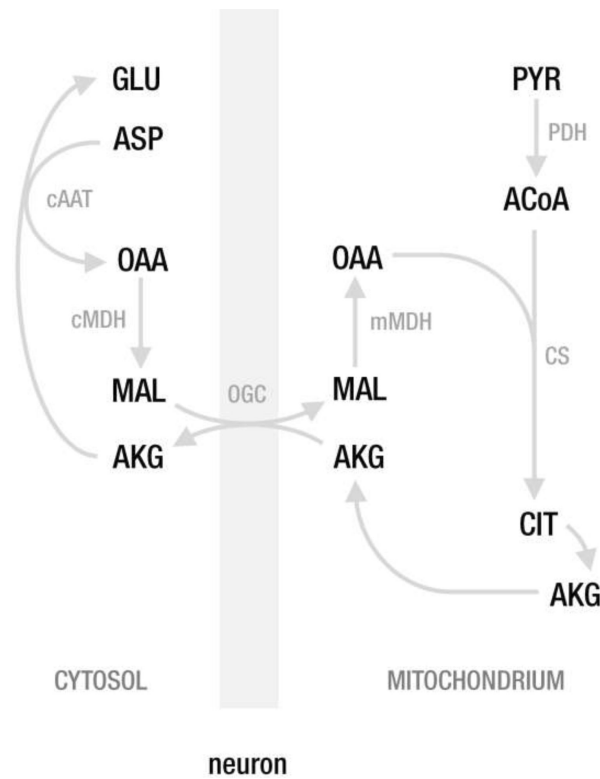


Figure 2. Proposed mechanisms for activity-dependent transient increase in [Glu] and decrease in [Asp] within the MAS context

A rise in pyruvate metabolism with net increase in acetyl-CoA determines a competition between mAAT and CS for intramitochondrial OAA. This in turn results in decreased substrate flow through AGC1. On the cytosolic side, the concentration of glutamate is increased and that of aspartate is decreased because cAAT remains active. As OGC does not remain without substrates, NADH can still be shuttled from the cytosol to the mitochondria. Importantly, in spite of interrupted flow through AGC1 this partial MAS preserves the ability to increase. Note that this mechanism occurs in parallel with the canonical MAS pathways (not shown).