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Review

CaMKII in cerebral ischemia

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Ischemic insults on neurons trigger excessive, pathological glutamate release that causes Ca^{2+} overload resulting in neuronal cell death (excitotoxicity). The Ca^{2+}/calmodulin (CaM)-dependent protein kinase II (CaMKII) is a major mediator of physiological excitatory glutamate signals underlying neuronal plasticity and learning. Glutamate stimuli trigger autophosphorylation of CaMKII at T286, a process that makes the kinase "autonomous" (partially active independent from Ca^{2+} stimulation) and that is required for forms of synaptic plasticity. Recent studies suggested autonomous CaMKII activity also as potential drug target for post-insult neuroprotection, both after glutamate insults in neuronal cultures and after focal cerebral ischemia *in vivo*. However, CaMKII and other members of the CaM kinase family have been implicated in regulation of both neuronal death and survival. Here, we discuss past findings and possible mechanisms of CaM kinase functions in excitotoxicity and cerebral ischemia, with a focus on CaMKII and its regulation.

Keywords: brain ischemia; excitotoxicity; glutamate; CaMKII; CaM kinases; stroke

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Introduction

Global cerebral ischemia is caused by a general loss of oxygen supply to the brain (during drowning, suffocation, or cardiac arrest). While the increased availability of automated external defibrillators has dramatically increased the survival rate after cardiac arrest, survivors may suffer from neuronal damage caused by the oxygen deficit in the brain. Despite increased demand for a neuroprotective treatment of global cerebral ischemia, no effective therapy has been developed to date. Focal cerebral ischemia (stroke) also involves decreased oxygen supply to parts of the brain. However, focal cerebral ischemia is caused by regional lack of blood supply after clotting or hemorrhage of blood vessels in the brain. The focal core area of the stroke is generally considered to be beyond rescue, however, neurons in the surrounding penumbra (where secondary cell loss occurs) are potential targets for therapeutic intervention[1] (Figure 1). Currently, the only available FDA approved pharmacological treatment is haemolytic therapy with tissue plasminogen activator (tPA). However, tPA is contra-indicated in hemorrhagic stroke, and by the time most patients receive diagnostic evaluation, tPA is no longer effective (and may even do more harm than good)[2–4]. Thus, less than 2% of stroke patients actually receive tPA[5], leaving a significant void in therapies that are more universally applicable for stroke treatment. Two independent studies recently implicated CaMKII as a promising drug target for post-insult neuroprotection[6, 7]. Here, we discuss the CaM kinase family and its possible involvement in the regulation of neuronal cell death, with a focus on CaMKII and its regulation.

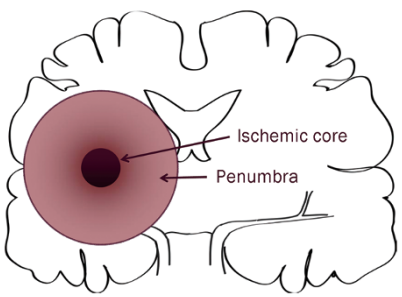

Figure 1. Ischemic core and penumbra after stroke. While neurons in the ischemic core are considered beyond rescue, neurons in the penumbra are potential targets for therapeutic intervention.

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Glutamate excitotoxicity causes neuronal cell death after cerebral ischemia

Glutamate is the major excitatory neurotransmitter in the mammalian brain. However, excessive glutamate also leads to neuronal cell death via "glutamate excitotoxicity", a process that involves Ca^{2+} overload[8] and that was first demonstrated in retinal neurons[9], and subsequently in the brain[10, 11]. Ischemic conditions trigger anoxic depolarization of neurons, which in turn triggers massive release of glutamate[1, 12, 13]. It is thought that the excitotoxic effect of such glutamate overstimulation causes much of the neuronal cell death seen following cerebral ischemia[13–16]. Additionally, excitotoxic glutamate signaling is thought to contribute to neuronal cell death after traumatic brain injury and in neurodegenerative diseases[16–18]. Excitotoxicity can cause neuronal death with apoptotic or necrotic appearance[19, 20], but both may involve similar signaling pathways despite a different morphological end state[14, 21, 22]. Overstimulation of most glutamate receptors can cause neuronal cell death, but the Ca^{2+} conducting NMDA-type glutamate receptors appear to be the most sensitive "death receptors"[23–26]: Even relatively brief (~5 min) application of glutamate or NMDA to cultured neurons can trigger signaling events that cause significant cell death within 24 h. Consequently, extensive efforts were made to develop NMDA-receptor inhibitors into stroke therapies, unfortunately without success[27, 28]. Alternative strategies include targeting signaling molecules downstream of the NMDA-receptor, such as CaMKII, which has been shown to mediate key effects of physiological NMDA-receptor stimulation in neuronal plasticity and learning and memory[29–31].

CaMKII and the CaM kinase family

CaM kinases (Figure 2) are a large family of Ser/Thr protein kinases that include kinases with broad substrate spectrum (*ie* multifunctional kinases such as CaMKI, CaMKII, and CaMKIV) and with high substrate selectivity [*ie* dedicated kinases such as myosin light chain kinases (MLCKs) and phosphorylase kinase (PhK)]. As the name implies, CaM kinases are generally activated by binding of Ca^{2+}/CaM to their regulatory region. However, several CaM kinases (including DAPK3 and AMPKs) lack a CaM-binding regulatory region, but are included in the CaM kinase family based on high homology of their core kinase domain. Regulation by Ca^{2+}/CaM does not automatically classify a kinase in the CaM kinase family. For instance, CaMKIII (now termed eEF2 kinase), which is also activated by Ca^{2+}/CaM, is not closely related to the other CaM kinases[32] and is instead grouped with the family of atypical protein kinases[33]. Like many other kinases (including PKA, PKB/Akt, and PKC), several CaM kinase family members require phosphorylation within the activation loop of their core kinase domain for full activity (including CaMKI T177, CaMKIV T196, and AMPKs T172). Interestingly, an upstream kinase that phosphorylates the activation loop of CaMKI, CaMKIV, and AMPK (as well as PKB/Akt, which is not a CaM kinase) is CaMKK, which is itself a Ca^{2+}/CaM-stimulated kinase[34]. However, other CaM kinases (including CaMKII and DAPKs) do not even have a phosphorylatable residue at the homologous activation loop position, even though their activity can be regulated by other phosphorylation events outside of their core kinase domains (such as T286 of CaMKII, which makes the kinase partially Ca^{2+}/CaM-independent[35–38], and S735 of DAPK1, which further enhances Ca^{2+}/CaM stimulated activity[39]).

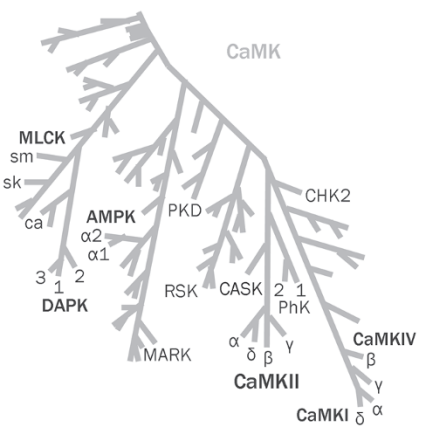

Figure 2. The CaM kinase family tree[33], with a limited number of example CaM kinases marked.

Functionally, DAPKs (death associated protein kinases) are associated with regulation of cell death[40], MLCKs (myosin light chain kinases) regulate smooth muscle contraction[41], and AMPKs (AMP activated kinases) are regulators of energy metabolism[42]. CaMKI, CaMKII, and CaMKIV have been implicated in various neuronal functions, including plasticity[30, 31, 43]. CaMKII comprises a family of closely related kinases, with four isoforms (α, β, γ, and δ) encoded by different genes, and alternative splicing gives rise to additional diversity[31]. At least one CaMKII isoform was found to be expressed in every cell type examined, with CaMKIIγ and CaMKIIδ being the most ubiquitous isoforms[44, 45]. CaMKIIα is almost exclusively expressed in brain, where it is also extremely abundant, making up more than 1% of total protein in some brain regions, such as the hippocampus[46]. Notably, the hippocampus, specifically its CA1 region, is also the brain area that is most sensitive to damage following global cerebral ischemia[47]. The hippocampus is important in memory formation, and the CaMKIIα knockout mice were the first knockout mice described to show impaired neuronal plasticity and learning[48, 49].

CaMKII structure and regulation

The relationship between CaMKII structure and regulation has been reviewed previously in detail[31]. However, there have since been several significant advances, especially regarding CaMKII structure[50–53] (Figure 3). CaMKII forms 12meric holoenzymes, with the C-terminal association domains forming a central hub and the N-terminal kinase domains radiating outwards like spokes or petals (Figure 3A). Between the core kinase domain and the association domain, the CaMKII

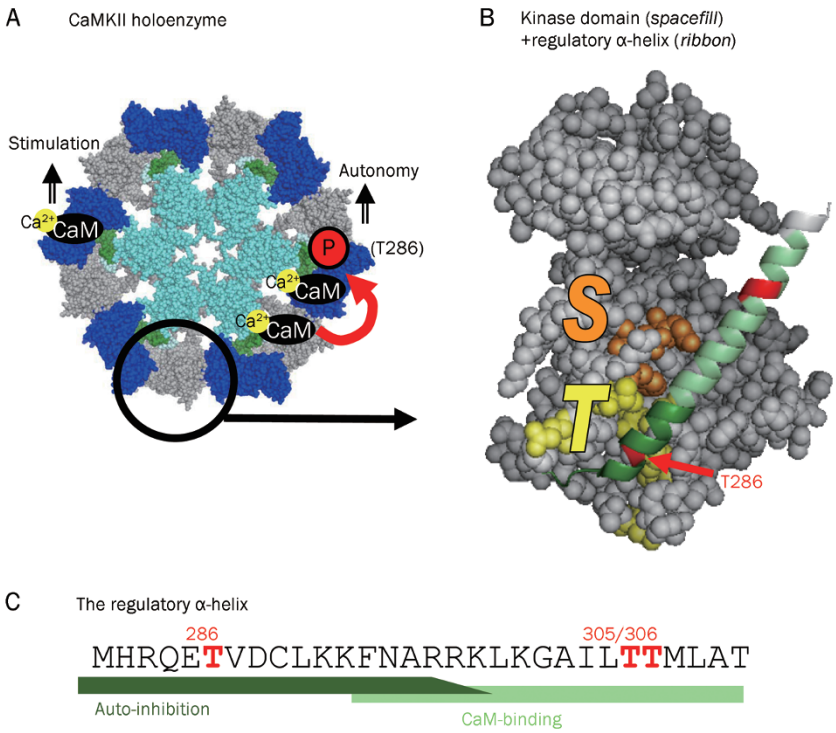

Figure 3. CaMKII structure[50, 51] and regulation. (A) CaMKII forms multimeric holoenzymes via C-terminal association domains (acqua). Each kinase domain (grey, dark blue) is stimulated separately by Ca^{2+}/CaM binding, but intersubunit autophosphorylation at T286 generates autonomous activity that persists even after dissociation of Ca^{2+}/CaM. (B) In the basal state, the regulatory α-helix (ribbon) interacts with the T-site (yellow) and prevents access to the substrate binding S-site (orange). Autophosphorylation sites (red) generate autonomous activity (T286) or prevent CaM binding (T305/306) and affect targeting. (C) The sequence of the regulatory α-helix. The regions contributing to CaM-binding and to auto-inhibition are marked.

subunits contain a Ca^{2+}/CaM-binding autoregulatory region followed by a variable region that is subject to extensive alternative splicing. The largest described splice variant is CaMKII βM (72 kDa)[54]; the dominant isoforms in brain are α (50 kDa) and β (60 kDa)[55, 56]. Holoenzymes can be homomeric or heteromeric (*ie* formed by subunits of the same or different isoforms)[54, 56–60], with a molecular weight of ~600–750 kDa and a diameter of ~20 nm[38, 50–52, 61, 62].

The α-helical autoregulatory region is responsible for holding CaMKII in an inactive state under basal conditions (Figure 3B). Its N-terminal part surrounding T286 interacts with the core kinase domain at a region termed the T286-binding site (T-site)[51, 63, 64]. When the autoregulatory region is bound to the T-site, access to the adjacent substrate binding S-site is blocked, thus preventing CaMKII activity (Figure 3B). Each kinase subunit within a holoenzyme is activated separately by direct binding of Ca^{2+}/CaM to the autoregulatory region ("stimulated activity"). This Ca^{2+}/CaM-binding displaces the autoregulatory region from the T-site, allowing the kinase to make other protein-protein interactions via the T-site (see below). Importantly, displacement of the autoregulatory region also allows access to the S-site, thereby activating the kinase. Additionally, releasing T286 from the T-site makes T286 accessible for auto-phosphorylation by neighboring subunits within the holoenzyme, provided these neighboring subunits are also activated with an exposed S-site[65, 66]. This auto-phosphorylation of T286 prevents complete re-association of the autoregulatory region with the T- and S-sites, leaving CaMKII partially active even after dissociation of Ca^{2+}/CaM ("autonomous activity")[35–37,67]. This autonomous activity has been described as a molecular memory mechanism and is indeed important for synaptic plasticity and learning[67, 68]. More recently, autonomous CaMKII activity has also been identified as a drug target for post-insult neuroprotection[6, 7].

An additional regulatory auto-phosphorylation at T305/306 can occur as an intra-subunit reaction and prevents subsequent Ca^{2+}/CaM binding[69–72] (Figure 3B). *Vice versa*, Ca^{2+}/CaM binding prevents this inhibitory auto-phosphorylation. Thus, efficient T305/306 auto-phosphorylation occurs only after generation of autonomous activity by T286 auto-phosphorylation and subsequent Ca^{2+}/CaM dissociation. Such a triple-phosphorylated CaMKII would be locked in a partially active state that cannot be further stimulated[67], but it would be completely inactivated only after selective T286 dephosphorylation.

Glutamate-induced translocation of CaMKII

Ca^{2+}-influx through NMDA-type glutamate receptors stimulates CaMKII activity and T286 auto-phosphorylation. Both are required for induction of long-term potentiation of synaptic strength[68, 73, 74], a form of synaptic plasticity underlying learning and memory[29, 30, 75]. Additionally, glutamate-induced Ca^{2+}-influx causes two forms of CaMKII translocation: (i) to post-synaptic sites[64, 76–78], and (ii) to extra-synaptic clusters[79–82]. Synaptic translocation occurs after physiological glutamate stimuli and is involved in synaptic plasticity[83, 84]. Several synaptic proteins have been described as binding partners for CaMKII, but the NMDA receptor subunit GluN2B (formerly known as NR2B) appears to be most important for CaMKII translocation to the synapse[64, 78, 85–89]. Extra-synaptic clustering has been described after pathological glutamate stimuli and ischemic conditions[79–82], and is likely mediated by

self-association of multiple CaMKII holoenzymes into large insoluble complexes[79, 90, 91], a process here termed aggregation. Both types of translocation require protein-interactions via the CaMKII T-site, specifically T-site binding to the GluN2B region around S1303[64, 85, 92] or the region around T286 of a CaMKII subunit within another holoenzyme[82, 90, 91]. Accordingly, both interactions require Ca^{2+}/CaM-binding, in order to make the T-site accessible. Both interactions are also enhanced by nucleotide. However, holoenzyme aggregation *in vitro* requires mimicking ischemic conditions, *ie* low ATP/ADP ratio and low pH. High ATP concentrations favor T286 auto-phosphorylation, which enhances CaMKII binding to GluN2B[64, 85], but inhibits CaMKII aggregation (as it inhibits interaction of T286 with the T-site)[91]. The molecular basis for requirement of a pH below 6.8 for CaMKII aggregation is unclear, but may involve protonation of His282, which is located at the hinge of the auto-regulatory α-helix (Figure 3B). CaMKII aggregation causes a reduction in the degree of activity that can be stimulated by Ca^{2+}/CaM[79], an effect also observed in brain after ischemia[93–95]. For this reason, it has been speculated that CaMKII aggregation may be a neuroprotective mechanism, as it would limit aberrant CaMKII activity during disregulated, pathological Ca^{2+} signaling[79]. However, the potential neuroprotective functions of CaMKII aggregation have yet to be determined.

Inhibitors of CaMKII

The traditional small molecule CaMKII inhibitors KN62 and KN93[96, 97] have proved to be very useful tools for studying CaMKII functions in cells, as they are membrane penetrating and their selectivity for CaMKII is relatively good[96–98]. However, the KN inhibitors cannot distinguish between CaMKII and CaMKIV[99]. Through an unusual affect on the scaffolding protein AKAP79/150[100], KN inhibitors can also inhibit PKC action at the synapse[101]. Maybe more importantly, KN inhibitors additionally affect voltage gated Ca^{2+} and K^+ channels[102, 103]. Another limitation is that both KN62 and KN93 are competitive with CaM and inhibit only stimulated but not autonomous CaMKII activity[6, 96, 97]. Peptide inhibitors derived from the CaMKII autoregulatory region, such as AC3-I or AIP[7, 104, 105], are generally thought to be more selective than the KN compounds. However, they can also inhibit other kinases, including PKC, MLCK, and PKD[106–108]. Additionally, some studies indicated a relatively low potency (IC_{50} of ~3 μmol/L)[109, 110].

More recently, an alternative was provided by CN21, a potent and selective CaMKII inhibitor[111]. CN21 is a 21mer peptide derived from the natural CaMKII inhibitory protein CaM-KIIN[112]. Peptides and even proteins can be made cell penetrating by fusion with sequence motifs such as ant/penetratin or tat[113–115]. In initial attempts to make CN peptides cell penetrating, ant fusion was used[116, 117]. However, it was subsequently shown that the ant/penetratin sequence directly binds CaM, an effect further enhanced by fusion with CN21[74]. Thus, while fusion of CN to ant added an additional CaMKII-inhibitory mechanism, selectivity for CaMKII was compromised. However, alternative fusion of CN21 to tat still allowed cell-penetration without the caveat of binding CaM[74]. The resulting tatCN21 is a potent (IC_{50} of ~50 nmol/L), selective, and cell penetrating peptide inhibitor of both stimulated and autonomous CaMKII activity[6, 74, 111]. Consistent with previous reports that tat fusion peptides can cross the blood brain barrier[26, 118], tatCN21 inhibited CaMKII functions in brain even after systemic application[6, 74].

CaMKII autonomy as a drug target for post-insult neuroprotection

Inhibiting stimulated and autonomous CaMKII activity with tatCN21 attenuated neuronal cell death induced by glutamate insult in primary cultures as well as in a mouse model of stroke (middle cerebral arterial occlusion; MCAO), even when administered significantly after the insult[6]. The longest post-insult time periods tested in this study were 6 h in hippocampal cultures, 1 h in cortical cultures, and 1 h *in vivo*[6]. A recent study independently confirmed post-insult neuroprotection by CaMKII inhibition, using both tatCN21 and tatAIP[7]. This study found significant neuroprotection in cortical cultures also when tatCN21 was administered 2 h after the insult[7]. By contrast, the traditional CaMKII inhibitor KN93 was neuroprotective only when administered during but not after the insult in both studies[6, 7]. During an insult, tatCN21, tatAIP, and KN93 can inhibit CaMKII activation and/or generation of autonomous activity. However, after the insult, tatCN21 and tatAIP but not KN93 can inhibit autonomous CaMKII activity that has already been generated. Thus, these findings indicated that autonomous CaMKII activity is the relevant drug target for post-insult neuroprotection by tatCN21. Indeed, overexpression of CaMKII wild-type or the constitutively autonomous T286D mutant (which mimics T286 phosphorylation) increased glutamate-induced neuronal death significantly more than overexpression of the autonomy-incompetent T286A mutant[6]. Consistent with these recent results[6, 7], neuroprotection by CaMKII inhibition had been described previously, although only for inhibition during or prior to excitotoxic insults[119–123]. Protection was seen in cortical cultures[6, 7, 119, 121], hippocampal cultures[6, 123] and retinal cells[120, 122]. However, other studies indicated an opposite effect, *ie* that abolishing CaMKII activity can promote neuronal cell death[124–128]. Some of these results may be explained by different death-inducing stimuli and different culture systems, as cerebellar granule cells and spiral ganglion cells depend on depolarization-induced Ca^{2+} signals for survival[126–128]. Additionally, different results using the inhibitor KN93 may depend on the balance between death- and survival-promoting signals mediated by CaMKII and CaMKIV, respectively[125]. Notably, however, genetic knock-out of CaMKIIα resulted in increased infarct size in a mouse model of stroke[124], the opposite effect from the observation after acute CaMKII inhibition with tatCN21[6]. This difference could be explained by developmental effects caused by the absence of CaMKIIα. Indeed, the CaMKIIα knock-out mice are epileptic[129], and hyper-excitability may contribute to higher susceptibility to ischemic insults. Additionally, inhibition of

CaMKII and complete removal of CaMKIIα protein may have profoundly different effects on glutamate-induced neuronal death. Thus, it will be interesting to compare CaMKII knock down by RNAi with CaMKII inhibition in neuronal cultures and to compare CaMKIIα knock-out mice with existing knock-in mice that carry the inactive CaMKIIα K42R mutation[130]. However, a recent study indicated that long-term (8–24 h) inhibition of CaMKII activity (without loss of CaMKII protein) is sufficient to increase vulnerability to subsequent excitotoxic insults[7], even though acute CaMKII inhibition during or after the insults reduced neuronal cell death (Table 1)[6, 7].

Table 1. Post-insult neuroprotection by CaMKII inhibitors[6, 7]. Tested were glutamate insults in hippocampal and/or cortical cultures, and a MCAO stroke model in mouse. (Stim, stimulated; Aut, autonomous).

Inhibitor	CaMKII activity blocked		Neuroprotection when applied		
	Stim	Aut	During insult	After insult	After stroke
KN93	x	–	x	–	?
tatCN21	x	x	x	x	x
tatAIP	x	x	x	x	?

Other CaM kinases in neuronal death and survival

CaMKII is not the only CaM kinase family member implicated in the regulation of neuronal survival and cell death. For example, CaMKIV activity has been described to be neuroprotective in multiple systems, likely by its phosphorylation and activation of the transcription factor CREB[127, 131–133]. CaMKI may have a similar effect, as it can activate Mek/Erk, which, in turn, also activates CREB[134]. CaMKK activity is also neuroprotective, as its inhibition by STO-609[135] increases neuronal cell death[6]. CaMKK is an upstream activator of at least two survival kinases, CaMKIV (see above) and Akt/PKB (which is not a member of the CaM kinase family)[136]. However, CaMKK is also an upstream activator of AMPK, a non-CaM-binding CaM kinase family member that has been implicated in promoting stroke-related neuronal death[137, 138]. Interestingly, acute treatment with the AMPK activator metformin during stroke insults increases neuronal death, while previous treatment with metformin before the insult reduces cell death[139]. This is similar to the phenomenon of ischemic preconditioning, in which previous mild insults can partially protect from the effect of subsequent stronger insults[140–143], indicating that AMPK may be part of a mechanism for this ischemic preconditioning. Thus, AMPK is also a possible target for stroke treatment, however, the effect of therapeutically relevant post-insult AMPK inhibition has not yet been tested[144].

Death-associated protein kinases (DAPKs) are another branch of the CaM kinases family associated with the regulation of cell death and survival[40]. DAPK1 has specifically been implicated in mediating excitotoxicity and stroke related death of neurons[145]. Interestingly, this required an interaction between DAPK1 and the NMDA-receptor subunit GluN2B[145] at the same site that also interacts with CaMKII[64, 92]. This raises the possibility that some of the neuroprotective treatments designed to target DAPK1 or CaMKII may act in part through effects on the other kinase; in fact, targeting both kinases with the same compound may be desirable for maximal therapeutic effect. However, molecular manipulations indicated that targeting each kinase separately is also neuroprotective independent from effects on the other kinase[6, 145]. Interestingly, manipulations that dissociate the scaffolding protein PSD95 from GluN2B also protect neurons from excitotoxic and stroke induced death[26, 146]. It has been proposed that this neuroprotection is due to the resulting dissociation of neuronal nitric oxide synthase (nNOS) from the NMDA-receptor[26, 146]. It will be interesting to test if this manipulation additionally indirectly affects GluN2B interaction with CaMKII and/or DAPK1.

What are the downstream targets for CaMKII after excitotoxic insults?

The mechanisms by which inhibition of autonomous CaMKII activity may mediate post-insult neuroprotection are currently unclear. Possible pathways by which CaMKII may participate in the regulation of neuronal death and survival are described below and summarized in Figure 4. Potentially death promoting effects of CaMKII activity include increase of AMPA-receptor single channel conductance by phosphorylating its GluR1 subunit at S831[147, 148]. This effect is especially pronounced for GluR1-homomeric AMPA-receptors[149], which are (in contrast to GluR2 containing channels) Ca^{2+}-conducting[150, 151], and could thus further promote death-inducing Ca^{2+} overload. Indeed, such Ca^{2+}-conducting AMPA-receptors have been implicated in ischemic injury[152]. CaMKII could also further increase the Ca^{2+} overload by facilitating or potentiating L-type voltage dependent Ca^{2+} channels (VDCCs) via their α or β subunits[153, 154]. Additionally, CaMKII can directly interact with connexin hemichannels[155], which are important in neuronal homeostasis and for neuron-glia communication and have been implicated in glutamate-induced cell death[156–158]. CaMKII activity may also contribute to neuronal cell death through phosphorylation of acid-sensing ion channels, which enhances the ischemia-induced activation of the ion channel[123]. Recently, CaMKII activity was also shown to be required for ischemia-induced shuttling of cytoplasmic polyadenylation element binding 4 (CPEB4) into the nucleus[159]. CPEBs regulate cytoplasmic polyadenylation and translation in neurons[160, 161], and some cross-talk between CPEB1 and CaMKII signaling has been described[162, 163]. CPEB4 knock down induces neuronal death, which can be rescued by re-expression of CPEB4 wild type, but not to the same extent by its nuclear export-incompetent mutant[159]. Thus, nuclear retention of CPEB4 by CaMKII activity may contribute to ischemia-induced neuronal death. Another protein that induces neuronal death upon nuclear shuttling is apoptosis inducing

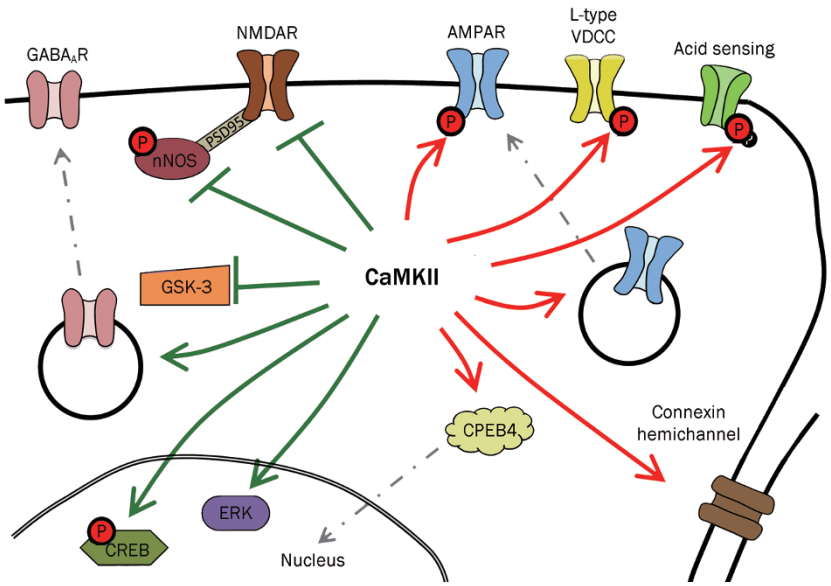

Figure 4. CaMKII downstream targets that may be involved in regulation of neuronal viability. CaMKII signaling may promote excitotoxic cell death (red) or neuronal survival (green). Activation (arrow) or inhibition (bar) of the downstream targets is indicated. Some but not all of these downstream effects are mediated by direct phosphorylation of the target.

factor (AIF)[164]. However, involvement of CaMKII in nuclear shuttling of AIF has not yet been tested.

On the other hand, several downstream effects of CaMKII activity could instead promote neuronal survival. Maybe most prominently, CaMKII can phosphorylate and inhibit nNOS[165–168], thereby reducing production of nitric oxide, which is neuro-toxic[14, 169–172]. CaMKII can also regulate several ion channels in a way that may afford neuroprotection from excitotoxic insults: It promotes desensitization NMDA-receptors[173], and it enhances surface expression of the inhibitory $GABA_A$-receptors[174, 175]. Additionally, CaMKII can activate pro-survival proteins such as Erk[117, 176] and CREB[177], inhibit death-promoting proteins such GSK-3[178] and Bad[127]. CaMKII can also promote neuronal survival by inhibiting HDAC5[126], a CaMKII substrate that inhibits the pro-survival transcription factor MEF[179]. These possible mechanisms may explain why the effect of CaMKII inhibition on cell survival can depend on the cell type and/or the death-inducing stimulus. Maybe more importantly, it may also explain why prolonged reduction of CaMKII activity increases vulnerability to subsequent excitotoxic insults[7, 124]. However, there is ample evidence that acute CaMKII inhibition protects hippocampal, cortical, and retinal neurons from glutamate excitotoxicity[6, 7, 119–123], consistent with the neuroprotection seen in a mouse model of stroke[6].

CaMKII regulation of apoptotic cell death: lessons from non-neuronal systems?

Apoptosis is a common mechanism of regulated cell death that involves caspase activation, nuclear condensation, and DNA fragmentation[180–183]. Ca^{2+} signaling and CaMKII have been linked to the regulation of apoptosis also in non-neuronal systems. However, as for the neuronal CaMKII targets discussed above, some of these links to apoptosis can promote cell death while others promote survival. CaMKII has been shown to be involved in mediating apoptosis induced by TNFα and UV irradiation[184] microcystein (phosphatase inhibitor)[185, 186], GW7845 (PPARγ agonist)[187], ER stress[188], TRAIL[189], and -in cardiac myocytes- by isoproterenol (β adrenergic agonist)[190, 191], ouabain (Na^+/K^+-ATPase inhibitor)[192], oxidative stress (induced by H_2O_2 or angiotensin II)[193–195], and cardiac ischemia[196–198]. In these cases, CaMKII inhibition attenuated apoptotic death. However, CaMKII inhibition has also been described to enhance apoptosis, indicating that CaMKII can also suppress apoptosis in other systems[199–204]. Anti-apoptotic CaMKII mechanisms included inhibition of caspase 2[199], enhancing expression of Bcl-xL[203], and promoting Akt-mediated inhibition of Bad[204]. Interestingly, CaMKII-mediated activation of Akt has been linked to both pro-[189] and anti-apoptotic[200, 201, 204] functions.

In the heart, CaMKII contributes to cell death in response to a variety of insults, and CaMKII inhibition consistently promoted cell survival[205] (see above). However, even in the heart, the situation is more complicated: While cytoplasmic CaMKII isoforms were consistently found to promote cell death, nuclear isoforms (*ie* CaMKII δ_B)[206] may instead promote survival[194, 207].

Clearly, the mechanisms by which CaMKII can regulate cell viability are complex and in need of further elucidation. However, the dual role for CaMKII in mediating both cell survival and death may not be that surprising after all, given that its principal activator, Ca^{2+}, is already well established to mediate both[208, 209]. Even Ca^{2+} signaling mediated by NMDA receptors can promote not only death but also survival[210, 211]. Generally, CaMKII appears likely to have pro-survival function in systems and situations in which Ca^{2+} is required for maintaining cell viability (such as activity-mediated neuronal survival). By contrast, in situations where Ca^{2+} acts instead as a trigger of death signaling, CaMKII is likely to be involved as a major mediator of cell death (such as in cerebral ischemia and exitotoxicity).

Concluding remarks and therapeutic potential

Recent results implicated that autonomous activity of CaMKII, induced by T286 auto-phosphorylation, provides a promising drug target for post-insult neuroprotection after cerebral ischemia and possibly other conditions involving glutamate excitotoxicity[6,7]. In the future, therapy development may include identification of selective small-molecule inhibitors of autonomous CaMKII. However, the peptide inhibitor tatCN21 itself also holds promise, as it was effective even after systemic delivery in an animal model[6, 74]. The toxicology for tatCN21 is still lacking, but no immediately obvious toxic effects were observed in mice. TatCN21 did interfere with learning in mice, which could be a potential contraindication for chronic treatment in humans[74]. However, for acute treatment after stroke, a temporary learning impairment would be more than acceptable, especially since tatCN21 did not interfere with memory storage or retrieval[74], and is thus unlikely to induce retrograde amnesia. Potency of tatCN21 is very reasonable, with an IC_{50} of ~50 nmol/L[74], and further improvements of potency may be possible. However, further studies are needed in order to elucidate the downstream mechanisms linking CaMKII to the neuronal cell death that is induced by ischemic conditions. Additionally, the window of therapeutic opportunity (at least 1 h in a mouse model of stroke[6]) needs to be evaluated more closely after different types of ischemic insults *in vivo*. After glutamate insults in culture, hippocampal neurons were significantly protected by tatCN21 application even 6 h after the insult[6], while protection of cortical neurons was only seen when tatCN21 was applied less than 3 h after the insult[7]. This difference may be caused by the type of glutamate insult (400 μmol/L for 5 min[6], versus 100 μmol/L for 60 min[7]). However, if the difference is instead inherent to the neuronal cell type, tatCN21 may have an even longer window of therapeutic opportunity after global cerebral ischemia, which particularly affects the hippocampus[47], compared to focal cerebral ischemia (stroke). Additionally, the therapeutic potential in other acute conditions involving glutamate excitotoxicity, such as traumatic brain injury, should be evaluated.

Financial disclosure

The University of Colorado is currently seeking patent protection for tatCN21, its derivatives, and its uses (including the ones outlined here).

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