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Invited review

The role of Ca^{2+}-calmodulin stimulated protein kinase II in ischaemic stroke – A potential target for neuroprotective therapies

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ABSTRACT

Studies in multiple experimental systems show that Ca^{2+}-calmodulin stimulated protein kinase II (CaMKII) is a major mediator of ischaemia-induced cell death and suggest that CaMKII would be a good target for neuroprotective therapies in acute treatment of stroke. However, as CaMKII regulates many cellular processes in many tissues any clinical treatment involving the inhibition of CaMKII would need to be able to specifically target the functions of ischaemia-activated CaMKII. In this review we summarise new developments in our understanding of the molecular mechanisms involved in ischaemia-induced CaMKII-mediated cell death that have identified ways in which such specificity of CaMKII inhibition after stroke could be achieved. We also review the mechanisms and phases of tissue damage in ischaemic stroke to identify where and when CaMKII-mediated mechanisms may be involved.

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The two main types of stroke are ischaemic (due to the occlusion of a cerebral blood vessel) and haemorrhagic (due to spontaneous haemorrhage either within the brain substance – intracerebral haemorrhage – or from arteries in the subarachnoid space around the brain – subarachnoid haemorrhage). Both cause acute onset of focal neurological injury from a vascular cause, but they differ in their mechanism of causation and tissue injury. Ischaemic stroke results in severe reduction of blood flow (hypoperfusion) within the territory of the occluded artery. Intracerebral haemorrhage causes physical disruption of neural tissue and chemical toxicity (e.g. iron) of substances from the haemorrhage. Ischaemic stroke accounts for the vast majority of clinical cases in developed communities and has been the focus of most of the research into the molecular and cellular mechanisms of brain damage following

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stroke and potential therapies to minimise brain damage. Several neuroprotective agents identified towards the end of the 20th century, which appeared to be effective in preclinical stroke models, failed to achieve success in clinical trials, leading to a general pessimism about the clinical viability of such approaches. However, meta-analyses of these studies showed that major deficiencies in experimental design of both the preclinical (Macleod et al., 2009) and clinical (Saver, 2013) trials can account for the failures. In addition, there is good clinical evidence for successful neuroprotection in global ischaemia following cardiac arrest and neonatal hypoxia-ischaemia (Bernard et al., 2002; Holzer et al., 2002; Shankaran et al., 2005). Therefore, there would appear to be great potential in pursuing molecular mechanisms, such as CaMKII, that are promising candidates for neuroprotective therapies in stroke.

Studies in multiple experimental systems that model ischaemic stroke (see below) show that inhibition of Ca^{2+}-calmodulin stimulated protein kinase II (CaMKII) can prevent 30–70% of ischaemia-induced cell death. This shows that CaMKII is a major mediator of ischaemia-induced cell death and suggests that CaMKII would be a good target for neuroprotective therapies in acute treatment of stroke. However, as CaMKII is widely expressed in most tissues, where it is involved in regulating many cellular processes (Skelding and Rostas, 2012; Hoffman et al., 2014), any clinical treatment involving the inhibition of CaMKII would need to be able to specifically target the functions of ischaemia-activated CaMKII. New developments in our understanding of the molecular mechanisms involved in ischaemia-induced CaMKII-mediated cell death have identified ways in which such specificity of CaMKII inhibition after stroke could be achieved.

1. Mechanisms of regulation of CaMKII

The CaMKII's are a family of multifunctional Serine(S)/Threonine(T) protein kinases normally activated by binding Ca^{2+}-calmodulin triggered by a rise in intracellular Ca^{2+} (Skelding and Rostas, 2012). In the nervous system, where it is expressed at levels that are 10–20 fold higher than in other tissues (Erondu and Kennedy, 1985), CaMKII is best known for its critical role in synaptic plasticity and learning (Giese et al., 1998; Rodrigues et al., 2004; Elgersma et al., 2002), but its importance in neuropathology, particularly in excitotoxicity, is increasingly being recognised (Churn et al., 2000; Mengesdorf et al., 2002; Lecrux et al., 2007; Gurd et al., 2008). The CaMKII family is encoded by 4 genes (α, β, δ, γ) producing > 30 isoforms, which regulate a range of biological functions in virtually every tissue (Jones, 2007; Munevar et al., 2008; Shin et al., 2008). All 4 genes are expressed in brain: α and β are expressed at higher levels than δ and γ, and α, which is the neuron-specific isoform, is expressed at highest concentrations in postnatal forebrain neurons where it can account for more than 1% of total protein (Erondu and Kennedy, 1985). All isoforms have a conserved domain structure (Fig. 1A): a C-terminal association domain that is responsible for multimerisation and contains the four main variable regions (V1-4), an N-terminal catalytic domain, and a regulatory domain in between that contains the calmodulin binding site and multiple conserved phosphorylation sites (Braun and Schulman, 1995). The holoenzyme consists of 12 subunits, which can be all the same isoform or include multiple isoforms, organised as 6 pairs of dimers arranged in a double rosette configuration (Rosenberg et al., 2005).

The functional roles of CaMKII are regulated by two mechanisms that modify each other: autophosphorylation and interactions with specific binding proteins. The binding proteins can be selective for particular CaMKII isoforms. In addition, although CaMKII is ubiquitously expressed, there can be tissue specific responses with respect to CaMKII because it is regulated by mechanisms involving binding proteins that are selectively expressed among tissues.

In its basal state each subunit is inactive because the autoinhibitory region blocks access to the active site (Fig. 1B). When the intracellular concentration of Ca^{2+} is elevated by normal or pathological stimulation, Ca^{2+}-calmodulin binds to its binding site in the regulatory domain (orange in Fig. 1). This causes the catalytic domain (green in Fig. 1) to swing away from the autoinhibitory region exposing the active site and allows CaMKII to phosphorylate substrates, including neighbouring subunits of the holoenzyme which can become phosphorylated at several sites. The functional roles of three of these sites – T286, T305/6 and T253[1] – have been elucidated:

(i) When T286 is phosphorylated it maintains the active site in the open position allowing CaMKII to phosphorylate its substrates even in the absence of bound Ca^{2+}-calmodulin – this is known as autonomously active CaMKII. Autophosphorylation at T286 occurs rapidly after activation by Ca^{2+}-calmodulin by an intra-holoenzyme mechanism that involves one subunit phosphorylating its neighbouring subunit both of which have bound Ca^{2+}-calmodulin. Phosphorylation at T286 enhances the affinity of Ca^{2+}-calmodulin binding to CaMKII greatly reducing its rate of dissociation (calmodulin trapping) (Meyer et al., 1992).

(ii) Phosphorylation at T305/6, which is within the calmodulin binding site (Fig. 1), can only be produced by autonomously active CaMKII once Ca^{2+}calmodulin has dissociated from its binding site. Consequently, T305/6 phosphorylation occurs as a delayed secondary response to a stimulus (Skelding et al., 2012). Once phosphorylation has occurred at T305/6, Ca^{2+}calmodulin can no longer bind to CaMKII to activate it, thereby making CaMKII insensitive to increases in intracellular Ca^{2+}.

(iii)Phosphorylation at the T253 site on CaMKII regulates CaMKII activity in a different way. In contrast to phosphorylation at T286 and T305/6, which directly alter CaMKII activity by inducing local alterations in the conformation of CaMKII, phosphorylation of T253 has no direct effect on CaMKII activity, but profoundly affects the targeting of CaMKII via interactions with specific binding proteins, with consequent functional effects on cell physiology (Migues et al., 2006; Skelding et al., 2010). The rate of phosphorylation of T253 is at least 10 times slower than that of T286 in vitro (Migues et al., 2006) whereas, in some tissues in vivo, the rate of stimulus induced T253 phosphorylation is equal to, or faster, than the rate of T286 phosphorylation (Skelding et al., 2012) highlighting the difference in behaviour caused by the local cellular microenvironment and/or the interaction of CaMKII with specific binding proteins.

Cells contain a variety of proteins capable of selectively binding non-phosphorylated or phosphorylated CaMKII. Phosphorylation at T286, T305/6 and T253 selectively alters (either enhances or inhibits) the binding of CaMKII to specific proteins changing the functional responses of CaMKII. Depending on the nature and cellular location of the binding protein, three kinds of functional effects can occur:

[1] Throughout this review, amino acid residues in CaMKII are numbered according their position in αCaMKII unless otherwise stated.

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