

Calcium Regulatory Protein, Regucalcin, may be a Key Molecule in Brain Disease

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Regucalcin was discovered in 1978 as a novel calcium-binding protein [1-4]. Regucalcin (RGN) and its gene (*rgn*) are identified in over 15 species of vertebrate and invertebrates consisting of regucalcin family [5]. Regucalcin plays a multifunctional role in cell regulation; maintaining of intracellular calcium (Ca^{2+}) homeostasis, suppressions of cell signaling, protein synthesis, nuclear deoxyribonucleic acid and ribonucleic acid synthesis and cell proliferation and apoptosis, and regulation of gene expression in various tissue and cell types [6,7]. Regucalcin has been proposed to play a pivotal role as a suppressor protein in signaling systems in maintaining of cell homeostasis [7]. The regucalcin gene is located on the X chromosome [8,9]. Interestingly, the map location for a growing number of diseases with a genetic basis is encompassed in human X chromosome, and these are known to include syndromic and non-syndromic forms of X-linked mental retardation and X-linked neuromuscular diseases [9]. Regucalcin is expressed in rat brain neuron, and it is decreased in the cerebral cortex and hippocampus of brain with aging [10,11]. There is growing evidence that regucalcin plays a pivotal role in brain neuronal cell regulation.

Neuronal Ca^{2+} homeostasis and Ca^{2+} signaling regulate multiple neuronal functions, including synaptic transmission, plasticity and cell survival [12]. Ca^{2+} -dependent biochemical processes have been implicated in mechanisms of neuronal plasticity like long-term potentiation, which is likely to play an important role in learning and memory [13]. These multiple functions of Ca^{2+} require fine regulation of its free intracellular concentration. Intracellular Ca^{2+} concentration in the neuronal cells of brain is regulated by various buffering and transport systems such as the membrane Na^+ - Ca^{2+} exchanges, the membranous Ca^{2+} -ATPase, Ca^{2+} -binding proteins, and intracellular Ca^{2+} uptake systems [14,15]. Ca^{2+} homeostasis undergoes subtle dysregulation in the physiologic aging. Disturbance of brain Ca^{2+} homeostasis may play a pivotal role in the causing of brain disease. Aging induces an increase in Ca^{2+} inflow by activating L-type voltage-sensitive Ca^{2+} channels and a decrease in Ca^{2+} efflux due to inhibiting the plasma membrane Ca^{2+} -pumping activity (Ca^{2+} -ATPase) in brain neurons, leading to a derangement of Ca^{2+} homeostasis [16]. Increased intracellular Ca^{2+} may be transported to the mitochondria and endoplasmic reticulum. In addition, the increase in the brain microsomal Ca^{2+} -sequestering system (Ca^{2+} -ATPase) with aging results in the microsomal calcium accumulation that is partly related to the brain toxicity by Ca^{2+} . Intracellular Ca^{2+} signaling is fundamental to neuronal physiology and viability. Because of its ubiquitous roles, disruptions in Ca^{2+} homeostasis are implicated in diverse disease processes and have become a major focus of study in multifactorial neurodegenerative diseases such as Alzheimer's disease [17]. The mitochondrial Ca^{2+} -ATPase activity and its calcium content are not altered with aging. The endoplasmic reticulum (microsomes) Ca^{2+} -ATPase activity is found to increase with aging, and the microsomal calcium accumulation is induced [18]. This enhancement may be related to Ca^{2+} -dependent factors in the cytoplasm of neuronal cells. Protein kinase C activity in the cytoplasm is increased with aging, and this enzyme may activate the microsomal Ca^{2+} -ATPase [18]. However, Ca^{2+} -calmodulin dependent protein kinase may not be involved in aging-induced increase in the

microsomal Ca^{2+} -ATPase activity [18]. Regucalcin has been found to have an inhibitory effect on brain microsomal Ca^{2+} -ATPase activity [10]. Brain microsomal Ca^{2+} -ATPase activity is not significantly altered by calmodulin, calbindin or S-100A protein, which are Ca^{2+} -binding proteins in brain [10]. The concentration of regucalcin in the brain tissues of young rats is about 5×10^{-9} M [19]. Brain microsomal Ca^{2+} -ATPase activity was decreased with 10^{-9} M which is a physiological concentration of regucalcin in the brain [10]. Meanwhile, regucalcin plays a role as an activator in the regulation of the mitochondrial Ca^{2+} -ATPase activity of rat brain tissues [20]. The addition of regucalcin (10^{-10} to 10^{-8} M) into the enzyme reaction mixture containing calcium chloride caused a significant increase in the mitochondrial Ca^{2+} -ATPase activity [20]. Thus, regucalcin may play a physiologic role in the regulation of intracellular Ca^{2+} homeostasis in rat brain. The concentration of regucalcin in the cerebral cortex and hippocampus of brain tissues is decreased with aging [19]. Suppressive effect of regucalcin on brain microsomal Ca^{2+} -ATPase activity has been found to weaken with aging [10]. Aging-induced elevation of brain microsomal Ca^{2+} -ATPase activity may partly be resulted from an attenuation of the suppressive effect of regucalcin on the microsomal Ca^{2+} -ATPase with aging, suggesting a pathophysiologic role of regucalcin in the regulation of brain intracellular Ca^{2+} homeostasis. Many functional proteins are degraded in the endoplasmic reticulum of brain neurons. Disturbance of Ca^{2+} homeostasis in the endoplasmic reticulum with aging may lead to an impairment of the degradation of modified proteins in brain neurons. The augmentation of modified proteins may promote cytotoxicity in the neurons.

Ca^{2+} /calmodulin-dependent protein kinases and protein kinase C play an important role in response of the cells to calcium signal in various cell types [21]. These protein kinases play a pivotal role in neuronal signaling processes [22]. Brain cytosolic Ca^{2+} -dependent protein kinase activity is raised with increasing age [23]. Regucalcin has been shown to have an inhibitory effect on Ca^{2+} /calmodulin-dependent protein kinase and protein kinase C activities in the cytoplasm of brain tissues [23] and neurons [24]. Suppressive effects of regucalcin on brain Ca^{2+} -dependent protein kinase activity may be weakened with increasing age [23]. Moreover, protein phosphatase plays an important role in intracellular signal transduction [25]. Regucalcin has been shown to have an inhibitory effect on Ca^{2+} /calmodulin-dependent protein phosphatase activity toward phosphotyrosine, phosphoserine

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and phosphothreonine in rat brain cytoplasm and neurons [21,26]. Calbindin and S-100A proteins, which are Ca^{2+} -binding protein in brain [11], have no effect on protein phosphatase activity in rat brain cytoplasm [26]. Regucalcin is localized in the microsomes of rat brain, and aging causes a decrease in the microsomal regucalcin levels [27]. Brain microsomal protein tyrosine phosphatase activity is increased with increasing age [27]. This enzyme activity is found to be increased in the presence of anti-regucalcin monoclonal antibody in the enzyme reaction mixture, indicating that endogenous regucalcin in brain microsomes may have a suppressive effect on the enzyme activity [27]. The decrease in regucalcin in brain microsomes with aging is partly related to the enhancement of microsomal protein tyrosine phosphatase activity in the brain of aged rats. Interestingly, regucalcin has been found to be decreased in the nucleus of rat brain with aging [28]. Brain nuclear protein tyrosine phosphatase activity is increased in the presence of anti-regucalcin monoclonal antibody in the enzyme reaction mixture and this effect is deteriorated in aged rats [28]. These findings may be a result of the decrease in brain nuclear endogenous regucalcin levels with increasing age [28]. Thus, regucalcin plays a suppressive role in the regulation of protein tyrosine phosphatase activity in the nucleus of rat brain. Protein phosphatase may be important in the regulation of proteins phosphorylated by protein kinases [29]. Regucalcin may have a role in the control of dephosphorylation of phosphorylated proteins due to inhibiting protein tyrosine phosphatase in both the cytoplasm and nucleus of rat brain. Phosphorylated proteins may be important in the regulation of brain neuronal cell functions which are related to long-term potentiation with learning and memory [30]. The reduction of endogenous regucalcin in rat brain nucleus with aging may lead to the disturbance of brain functions.

Regucalcin has also been demonstrated to have a protective action against oxidative damage, without influencing antioxidant enzyme status in brain [31]. Moreover, regucalcin has been found to inhibit nitric oxide (NO) synthase activity in the cytosol of rat brain tissues [32]. NO synthase is activated by Ca^{2+} /calmodulin which relates to Ca^{2+} signaling. NO, which plays a role as a messenger or modulator molecule in various cell types, is produced by NO synthase [33]. Overproduction of NO may lead to the damage of various cells. Regucalcin may have a suppressive effect on overproduction of NO in the cells and may have a protective effect on NO-induced damage of cells. Regucalcin may regulate brain functions, which are related to NO, supporting the view that regucalcin plays a role as the regulatory protein in brain function. Disruptions in Ca^{2+} homeostasis are implicated in diverse disease processes and have become a major focus of study in neurodegenerative diseases such as Alzheimer disease [17]. A hall mark of Alzheimer disease is the excessive production of β -amyloid and its massive accumulation in amyloid plaques. Altered Ca^{2+} signaling accelerates amyloid-beta formation, whereas amyloid-beta peptides, particularly in soluble oligomeric forms, induce Ca^{2+} disruptions [17]. A degenerative feed-forward cycle of toxic amyloid-beta generation and Ca^{2+} perturbations results, which in turn can spin off to accelerate more global neuropathological cascades, ultimately leading to synaptic breakdown, cell death, and devastating memory loss [17]. The amyloid hypothesis of Alzheimer's disease posits that the fundamental cause of Alzheimer's disease is the accumulation of the peptide amyloid-beta in the brain [34]. This hypothesis has been supported through observations that genetic defects in amyloid precursor protein and presenilin increase amyloid β production and cause familial Alzheimer's disease [34].

Regucalcin has also been demonstrated to be involved in Parkinson's disease. Interestingly, the proteomic analysis ever of post-mortem locus

ceruleus tissue of six pathologically confirmed Parkinson's disease patients, and six age- and gender-matched non-neurological controls showed that several individual proteins are identified that have hitherto not been associated with Parkinson's disease, such as regucalcin, which plays a role in maintaining intracellular Ca^{2+} homeostasis, and isoform 1 of kinectin, which is involved in transport of cellular components along microtubules [35]. In total 2495 proteins are identified, of which 87 proteins were differentially expressed in the locus ceruleus of Parkinson's disease patients compared with controls [35]. The majority of these differentially expressed proteins are known to be involved in processes that have been implicated in the pathogenesis of Parkinson's disease, including mitochondrial dysfunction, oxidative stress, protein misfolding, cytoskeleton dysregulation, and inflammation [14]. Moreover, the neurofibrillary tangles (NFTs) formed by the accumulation of abnormal tau filaments have been shown to be involved in Alzheimer's disease brain degeneration. A tau transgenic mouse (pNSE/htau23) model was used to monitor changes in protein levels and to search for novel biomarker candidates suitable for the early diagnosis of Alzheimer's disease before onset of clinical symptoms [36]. Plasma samples from 2-month (asymptomatic) and 4-month (symptomatic) tau transgenic mice were compared to the control group by 2-dimensional gel electrophoresis coupled with liquid chromatography-tandem mass spectrometry. Three proteins, ATP synthase, adenosine kinase and regucalcin showed significantly decreased levels in the plasma of tau transgenic mouse, which was further confirmed by Western blotting [36]. This study suggests that regucalcin could be used as candidate biomarkers for early diagnosis of Alzheimer's disease in combination with previously discovered protein biomarkers. Thus, regucalcin may play an important role in revelation of brain disease including Alzheimer's and Parkinson's disease.

As described above, regucalcin has been shown to play a multifunctional role as a regulatory protein in cell signaling in brain neuronal cells [6,7]. In this aspect, regucalcin has been demonstrated to play in the regulation of Ca^{2+} homeostasis and Ca^{2+} signaling in brain neurons; the protein reveals suppressive effects on microsomal Ca^{2+} pump activity, Ca^{2+} -dependent protein kinases, protein phosphatases and NO synthase activities in the brain of rats. Brain regucalcin concentration is decreased with aging, and the regulatory effects of regucalcin in brain neuronal cell functions are weakened with increasing age. The decrease in regucalcin gene expression with aging may be implicated to leading to neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Regucalcin may be a target molecule in brain diseases with aging. Development of further studies will be expected.

Author Contribution and Disclosures

The author has no conflicts of interest.

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