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Chronic high levels of the RCAN1-1 protein may promote neurodegeneration and Alzheimer disease

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Abstract

The *RCAN1* gene encodes three different protein isoforms: RCAN1-4, RCAN1-1L, and RCAN1-1S. RCAN1-1L is the RCAN1 isoform predominantly expressed in human brains. RCAN1 proteins have been shown to regulate various other proteins and cellular functions, including calcineurin, glycogen synthase kinase-3 β (GSK-3 β), the mitochondrial adenine nucleotide transporter (ANT), stress adaptation, ADP/ATP exchange in mitochondria, and the mitochondrial permeability transition pore (mtPTP). The effects of increased *RCAN1* gene expression seem to depend both on the specific RCAN1 protein isoform(s) synthesized and on the length of time the level of each isoform is elevated. Transiently elevated RCAN1-4 and RCAN1-1L protein levels, lasting just a few hours, can be neuroprotective under acute stress conditions, including acute oxidative stress. We propose that, by transiently inhibiting the phosphatase calcineurin, RCAN1-4 and RCAN1-1L may reinforce and extend protective stress-adaptive cell responses. In contrast, prolonged elevation of RCAN1-1L levels is associated with the types of neurodegeneration observed in several diseases, including Alzheimer disease and Down syndrome. RCAN1-1L levels can also be increased by multiple chronic stresses and by glucocorticoids, both of which can cause neurodegeneration. Although increasing levels of RCAN1-1L for just a few months has no overtly obvious neurodegenerative effect, it does suppress neurogenesis. Longer term elevation of RCAN1-1L levels (for at least 16 months), however, can lead to the first signs of neurodegeneration. Such neurodegeneration may be precipitated by (RCAN1-1L-mediated) prolonged calcineurin inhibition and GSK-3 β induction/activation, both of which promote tau hyperphosphorylation, and/or by (RCAN1-1L-mediated) effects on the mitochondrial ANT, diminished ATP/ADP ratio, opening of the mtPTP, and mitochondrial autophagy. We propose that RCAN1-1L operates through various molecular mechanisms, primarily dependent upon the length of time protein levels are elevated. We also suggest that models analyzing long-term *RCAN1* gene overexpression may help us to understand the molecular mechanisms of neurodegeneration in diseases such as Alzheimer disease, Down syndrome, and possibly others.

Keywords

RCAN1; Neurodegeneration; Oxidative stress; Alzheimer disease; Mitochondria; Free radicals

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Introduction

The RCAN1¹ proteins are encoded by the *RCAN1* (regulator of calcineurin) gene [1], which was previously known by a wide variety of names, including *Adapt78*, *DSCR1*, and *MCIP1*. At least three RCAN1 isoforms have been described in various organisms so far: RCAN1-4, RCAN1-1L, and RCAN1-1S [2,3]. The -1L and -1S postscripts for RCAN1-1 refer to the long and short length forms of RCAN1-1, respectively. The *RCAN1* gene is transiently induced during cell adaptation to oxidative/calcium stress [4]. It can also be induced by other stresses, including biomechanical stress [5] and psychological stress [6]. It has actually been shown that very transient *RCAN1* induction, lasting only 2–6 h, can be protective against acute stress [7,8]. However, chronic *RCAN1* overexpression is associated with Down syndrome and Alzheimer disease [9–13], and we have hypothesized that chronic *RCAN1* induction can have harmful effects and may be one factor that promotes the initiation and/or progression of neurodegenerative disorders [3,6,9,14–16]. It has also been shown that upregulation of RCAN1's can have adverse effects in cultured neuronal cells [17,18] as well as on *Drosophila* in vivo [19,20] and in mice [21]. The *Drosophila* RCAN1 homolog has been shown to alter brain function and regulate learning [19,20], whereas overexpression of mouse *RCAN1* causes hippocampal deficits that alter learning and memory in a pattern reminiscent of Down syndrome [21]. Our recent data also show that elevated levels of RCAN1-1L for prolonged periods can cause severe metabolic disturbances due to mitochondrial dysfunction and autophagy [22]. Using two neuronal cell models, ST14A developed from rat striatal neurons and ENStem developed from human neuronal progenitor cells, we found that RCAN1-1L does not have a significant effect on cell division but it has a cumulative negative effect on cell survival. Thus, it is important to keep in mind that RCAN1's can have opposing effects depending on which exact isoforms are involved and the length of time for which any isoform is induced.

The RCAN1 protein isoforms: RCAN1-1L, RCAN1-1S, and RCAN1-4

RCAN1 was independently identified in several laboratories at about the same time. In our laboratory, it was discovered as *Adapt78*, an oxidative stress-protective factor, in a differential display screen of mRNA's preferentially expressed in cells that had been transiently (and reversibly) adapted to low-dose hydrogen peroxide treatment [4,7,8]. Identification of the *Adapt78* gene (now called the *RCAN1* gene) was part of a larger project to identify new stress-adaptive genes and proteins. We now know that we had actually discovered the gene encoding the RCAN1-4 protein, whose levels in most tissues, including brain, are normally very low. *RCAN1-4* is, however, a highly stress-responsive gene and both transcription and translation levels can increase manifold in stress adaptation [4]. Levels of RCAN1-1 proteins also increase transiently and contribute to stress adaptation. However, RCAN1-1 protein levels do not increase as much as do RCAN1-4 protein levels and, unlike RCAN1-4 that is induced through mRNA transcription, they seem to be elevated by translational activation [17,23]. During transient adaptation to oxidative stress, a number of protective proteins are thought to be activated by phosphorylating kinases. We have proposed [4,7,8] that to prevent excessive activation, the phosphatase calcineurin dephosphorylates these same protein targets, thus acting as an “off switch” for several

adaptive responses, although a direct test of this hypothesis has not yet been performed. We propose that RCAN1-4, and to a lesser extent RCAN1-1 (simply because RCAN1-4 is so much more stress responsive than is RCAN1-1), functions by preventing calcineurin from dephosphorylating its targets and turning off the adaptive response; in other words, by inhibiting calcineurin, RCAN1-4 and RCAN1-1 may ensure a longer, stronger, and more effective stress-adaptive response [4,7,8].

Transient, and readily reversible, increased synthesis of RCAN1-4 and RCAN1-1 during stress adaptation does not result in calcineurin dysregulation because RCAN1-4 and RCAN1-1 levels increase for only a few hours, during the initial stages of adaptive responses [4,7,8]. We very quickly began to wonder, however, about what might be the potentially deleterious effects of chronic *RCAN1* over-expression. The discovery that basal levels of RCAN1-1 protein are much higher than those of RCAN1-4 in most human tissues (including brain), and the finding that RCAN1-1 levels are chronically elevated even further in Alzheimer disease [9,10,15], led us to focus more on the possible connection between RCAN1-1 and neurodegeneration. In addition, both Estivill's group [12] and our own [9] found an association between Down syndrome and high intracellular levels of RCAN1-1 protein. Because Down syndrome patients suffer an early and aggressive onset of Alzheimer disease, this finding served to intensify our interest in a possible causal mechanistic link between chronically high synthesis of RCAN1-1 and the initiation or progression of neurodegenerative disorders.

Stress may lead to neurodegeneration by inducing RCAN1-1L

A variety of stresses have been associated with neurodegeneration. Psychological stress is one of the most common stresses in life, and it may significantly contribute to neurodegeneration. For example, it has been shown that elderly individuals subjected to psychological stress are more likely to develop Alzheimer disease than are age-matched, nondistressed individuals [24]. Using rodent models, it has been shown that stress induced by chronic physical restraint (immobilization) induces an atrophy of apical dendrites in hippocampal CA3c neurons [25], suppresses neurogenesis in the dentate gyrus [26], and alters dendritic morphology of prefrontal cortical neurons that are accompanied by impairments in the performance of cognitive tasks [27]. Findings that psychological stress can suppress neurogenesis in the dentate gyrus were also confirmed using the tree shrew model [28], which is more closely related to primates. Our data show that psychological stress can induce RCAN1-1L protein levels [6] and, because induction of RCAN1-1L can lead to cell death, we hypothesize that RCAN1-1L may be at least partially responsible for stress-induced neurodegeneration.

Similar to stress, RCAN1-1L has also been found to suppress adult neurogenesis and the density of dendritic spines in the hippocampus of mice [21], which is in good agreement with our hypothesis that stress may, in part, contribute to compromised brain functions by inducing chronically high levels of RCAN1-1L synthesis.

Stress-induced production of glucocorticoids may exacerbate neurodegeneration and Alzheimer disease by inducing RCAN1-1L

RCAN1-1L levels can be specifically increased by glucocorticoids [29,30], whose production is known to be stimulated by multiple stresses. High glucocorticoid levels can lead to neurodegeneration, and we hypothesize that stress-induced production of glucocorticoids may actually cause neurodegeneration by elevating RCAN1-1L. Glucocorticoids are stress-inducible hormones that carry out a number of important functions, including adaptation to stressful conditions. However, it has been demonstrated in animals and humans that excessive levels of glucocorticoids can cause neurodegeneration. For example, in adult rodents, glucocorticoid hypersecretion is associated with reduced volume of the cingulate cortex [31], as well as memory impairment and hippocampal atrophy [32]. Chronic glucocorticoid exposure induces amyloid β pathology in aged monkeys [33]. Finally, in humans, levels of plasma glucocorticoids negatively correlate with hippocampal volume and memory [34]; glucocorticoids have a depressing effect on axonal transport in prefrontal neurons [35]; glucocorticoid levels are higher in the hippocampus of Alzheimer disease patients than in age-matched controls [36]; and chronic glucocorticoid treatments seem to worsen the cognition of people with Alzheimer disease [37]. In a mouse model of Alzheimer disease, glucocorticoids increase amyloid β and tau pathology [38].

Despite a mounting body of evidence that glucocorticoids can contribute to neurodegeneration, the molecular mechanisms underlying this process are not well understood. It has been shown that expression of RCAN1-1 mRNA, which encodes the RCAN1-1L protein, is induced by glucocorticoids, but RCAN1-4 mRNA is not induced by glucocorticoids [29,30]. Interestingly, glucocorticoid-induced RCAN1-1 mRNA expression occurs even when protein synthesis is inhibited, indicating that RCAN1-1 mRNA is directly induced by glucocorticoids, rather than through the synthesis of signaling proteins or peptides. Thus, we propose that glucocorticoids may lead to neurodegeneration by increasing RCAN1-1L levels.

Possible mechanisms by which RCAN1-1L may cause neurodegeneration and Alzheimer disease

Several mechanisms by which RCAN1's can harm cells are possible, but they are still poorly understood (Fig. 1). The first function of RCAN1 proteins to be demonstrated was binding and regulation of calcineurin activity [12,39–41]. It soon became clear, however, that like many proteins, RCAN1's could have other functions. We found, for example, that RCAN1's can increase the levels of active GSK-3 β kinase protein [3]. However, the mechanism by which RCAN1's can induce GSK-3 β is unknown, and it is possible that they may activate GSK-3 β through calcineurin: in this model, RCAN1's would inhibit calcineurin, which would then lead to reduced dephosphorylation of GSK-3 β and its activation. The *Drosophila* homolog of RCAN1, called nebula, can bind to the mitochondrial ANT and regulate its ADP/ATP transporting activity [42]. In addition to facilitating ADP/ATP exchange through the mitochondrial membrane, ANT can carry out another important function—it can form the mitochondrial permeability transition pore (mtPTP), which is a key component of certain

forms of apoptotic and necrotic cell death [43,44]. In fact, ANT is currently considered to be one of the main components of the mitochondrial permeability transition pore [43,44]. Our studies demonstrate that RCAN1-1L can indeed regulate mammalian mtPTP [22]. Because mtPTP opening has been suggested to initiate autophagy and mitochondrial degradation [45,46], we tested this possibility and discovered that high levels of RCAN1-1L synthesis lead to induction of autophagy and significant loss of mitochondrial mass.

Thus, current data suggest that RCAN1's may contribute to neurodegeneration through the following pathways shown graphically in Fig. 1: (a) RCAN1's can inhibit calcineurin and increase GSK-3 β activity, both of which can increase cellular levels of hyperphosphorylated tau protein, which may lead to the formation of neurofibrillary tangles and neurodegeneration. We have already begun to test this hypothesis and find that prolonged high levels of RCAN1-1L can lead to accumulation of phosphorylated tau in the mouse model [6]; (b) RCAN1's can lower ADP/ATP exchange and ATP levels and cause increased mitochondrial production of superoxide and its dismutation product, hydrogen peroxide, which can cause cell damage [47]; (c) RCAN1's can bind to ANT and induce mtPTP opening that signals apoptosis, and it has been shown that RCAN1's can facilitate neuronal apoptosis in cell culture [18]. It is obvious that alteration of either the ADP/ATP transporter or the mtPTP may alter mitochondrial functions. It is also known that inhibition of calcineurin can alter mtPTP [48,49]; thus, it could be thought that all three of the above pathways might potentially alter mitochondrial functions. Of note, one study has suggested that *RCAN1* effects on mitochondria are independent of calcineurin [42], which would leave us with just two likely pathways (mtPTP opening and decreased ATP levels) resulting in the mitochondrial defects shown in Fig. 1. However, this claim may not be completely convincing because it was based solely on succinate dehydrogenase (SDH) staining results, rather than on the more standard SDH and cytochrome *c* oxidase costaining technique to detect nonfunctional mitochondria.

Conclusions

Although the damaging role of chronically high levels of RCAN1's is well established in *Drosophila* and cell culture models, causal roles for RCAN1's in mammalian neurodegeneration have not yet been demonstrated. Several controversial reports regarding the effects of RCAN1's in mouse brains have been made [50–52]. Unfortunately, these reports did not consider important factors such as the age of the animals or which RCAN1 isoform was actually overexpressed, which makes the interpretations of such investigations either incomplete or inconclusive. Studies that carefully consider such factors will, clearly, have to be conducted. As it may take several decades for some brain conditions to develop, we suggest that the time factor should be given special consideration.

Worldwide “population graying” is expected to dramatically increase the number of cases of Alzheimer disease and other neurodegenerative disorders. We appreciate that this expected increase in human suffering, and the large financial burden it will place on society, is fueling a major effort to find answers quickly, and we certainly sympathize with this imperative. One cannot escape from the fact, however, that Alzheimer disease-related neurodegeneration actually takes several decades to develop. It is not yet clear whether

transgenic animals that develop neurodegeneration within a few months will actually facilitate major breakthroughs in explaining or treating the disease. As studies of RCAN1-1L illustrate, ignoring the time factor in disease development and progression could be a mistake. As indicated at the start of this review, brief and transient increased RCAN1-1 synthesis can be protective, and high levels of RCAN1-1L for a few months have no obvious effect on neurodegeneration (although they suppress neurogenesis), whereas prolonged elevated RCAN1-1L levels lead to neurodegeneration. Thus, new models of neurodegeneration may be required to understand Alzheimer disease, and models analyzing RCAN1-1L over time should be considered.

Abbreviations

RCAN1	regulator of calcineurin 1
GSK-3β	glycogen synthase kinase-3 β
ANT	(mitochondrial) adenine nucleotide transporter
mtPTP	mitochondrial permeability transition pore

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Fig. 1.

Possible mechanisms by which long-term stress may cause neurodegeneration via chronic RCAN1 induction. Multiple forms of chronic stress can induce prolonged synthesis of RCAN1 proteins at high levels. Although both RCAN1-1 and RCAN1-4 protein levels can be increased by stress, the mechanisms by which protein levels are elevated seem to be different, and only RCAN1-1 is induced by glucocorticoids [29,30]. Chronically elevated levels of RCAN1-L cause long-term inhibition of calcineurin and increased activity of GSK-3 β , both of which can lead (over time) to the accumulation of hyperphosphorylated tau protein. The accumulation of hyperphosphorylated tau may (eventually) cause the formation of neurofibrillary tangles, which promote neurodegeneration. On the other hand, RCAN1-1L can bind to the mitochondrial adenine nucleotide translocator (ANT) and inhibit its ADP/ATP transporting activity, which can lead to reduced cellular ATP levels. RCAN1-1L binding to ANT can also cause opening of the mitochondrial permeability transition pore (mtPTP). Both mtPTP opening and decreased ATP levels can initiate mitochondrial autophagy ("mitophagy") and may even lead to apoptotic and/or necrotic cell death.