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T-type Ca^{2+} channels

New players in the aging brain

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Perhaps one of the most remarkable features of T-type calcium channels is their low-threshold of activation that makes these channels important candidates for calcium entry near the resting membrane potential of neurons. Hence, they mediate low-threshold burst discharges that occur during different forms of neuronal rhythmogenesis, but play also important roles in sensory transmission, as well as hormone and neurotransmitter release. Additionally, they have been implicated in an increasing number of neuronal pathologies including neuropathy, autism spectrum disorders and some forms of epilepsy. More recently, an implication of T-type calcium channel in the processing of Amyloid Precursor Protein was documented, with possible implication in the pathogenesis of Alzheimer's disease.

Neuronal calcium (Ca^{2+}) signaling is of critical importance as it participates in a plethora of cellular processes including membrane excitability, gene expression, synaptic transmission, synaptogenesis, cell death and survival, but also neuronal processes underlying learning and memory. In order to make use and regulate the amplitude, duration and subcellular localization of the Ca^{2+} signal, cells have developed a complex machinery, the so-called "Ca^{2+} signaling toolkit," comprised of ion channels, pumps, and exchangers both in the plasma membrane and in the membranes of intercellular organelles (endoplasmic reticulum, mitochondria, Golgi apparatus and nucleus), but also Ca^{2+} binding proteins and transcriptional factors that altogether coordinate neuronal Ca^{2+} signaling and homeostasis.[1] It is thus not surprising that alteration of the Ca^{2+} signaling machinery, causing either a loss or a gain of function, can lead to serious chronic neuronal disorders. Therefore, despite major intrinsic differences in the pathogenesis of neurodegenerative diseases, alteration in Ca^{2+} homeostasis has emerged as a common underlying mechanism in Parkinson's disease, Huntington's disease, spinocerebellar ataxias, amyotrophic lateral sclerosis or Alzheimer's disease (AD). Whereas the molecular mechanisms responsible for alterations in neuronal Ca^{2+} signaling in the aging brain are not clearly understood, numerous studies have reported age-related changes in the expression and/or activity of some of the key players of the Ca^{2+} machinery, often associated with decreased synaptic plasticity, and in some cases with progressive neuronal loss. Hence, an increased activity of L-type voltage-gated Ca^{2+} channels has been reported in aged hippocampal neurons.[2,3] In parallel, elevated intracellular Ca^{2+} release from the endoplasmic reticulum through ryanodine receptors (RyRs) has been observed,[4] possibly via enhanced Ca^{2+}-induced Ca^{2+} release (CICR) as consequence of increased Ca^{2+} influx through L-type Ca^{2+} channels.[5] Additionally, decreased plasma membrane Ca^{2+}-ATPase activity and Ca^{2+} extrusion,[6] as well as diminished Ca^{2+} buffering capacity of the aged neuron (decreased SERCA pump activity[7] and mitochondrial Ca^{2+} sink[8] have been reported, leading to an overall intracellular free Ca^{2+} overload. Consistent with the Ca^{2+} theory of the aging brain, pharmacological regulation of Ca^{2+} homeostasis using either Ca^{2+} chelators[9] or Ca^{2+}

channel inhibitors[10] have shown beneficial effects on age-related cognitive declines. In a recent study published in *Neurobiology of Aging*,[11] Rice et *al*., have examined the possible implication of new Ca^{2+}-related proteins in the aging brain. The results of this study indicate an age-dependent alteration in the expression of $Ca_v3.1$ T-type Ca^{2+} channel. More importantly, the authors demonstrate that T-type Ca^{2+} channels are functionally coupled to the proteolytic processing of Amyloid Precursor Protein (APP), one of the key suspects in the pathogenesis of Alzheimer's disease.[12]

In a first set of experiments, the authors investigated age-related changes in the expression of some of the Ca^{2+}-related genes. Microarray analysis from human brain samples revealed a significant decrease of about 45% in the expression of *CACNA1G*, that encodes the low-voltage-activated $Ca_v3.1$ T-type Ca^{2+} channel, in aged non-diseased (74–95) compared with young non-diseased (20–59) individuals. This drop was observed in various brain regions supporting cognitive functions, including the entorhinal cortex, the hippocampus, the posterior cingulate gyrus as well as the superior frontal gyrus. Interestingly enough, a further decrease in *CACNA1G* expression was observed in AD aged-patients compared with control aged-individuals in the hippocampus, a region well known to play an important role in the formation of new memories, but also one of the first regions in the brain to suffer damage in AD. Consistent with a drop in *CACNA1G* expression in the aging brain, western blot analysis of Cav3.1 channel performed on 3xTg-AD mouse brain (a genetic AD mouse model[13]) showed a significant decrease in channel expression with aging. However, a similar decrease in $Ca_v3.1$ channel expression was also observed in wild-type animals, suggesting that alteration in $Ca_v3.1$ channel expression is mostly related to aging, regardless of the development of AD. In order to evaluate if alteration in $Ca_v3.1$ channel expression observed in the aging brain contributes to the development of AD, the authors analyzed the functional implication of the channel in the processing of that is believed to play a critical role in the pathogenesis of AD. It is well established that proteolytic cleavage of APP by the β-secretase (also known as β-site APP cleaving enzyme-1 BACE1) produces a soluble form of APP ($sAPP_\beta$) and a 99-amino acid C-terminal fragment (C99) that remains anchored in the plasma membrane. Subsequent processing of C99 by a γ-secretase generates the so-called amyloid-β peptide (Aβ), mainly consisting of a 40-amino acid peptide ($A\beta_{40}$) and the hydrophobe 42-amino acid product ($A\beta_{42}$) that tends to form fibrils, and constitutes the main component of extracellular senile plaques observed in AD brains. In contrast, proteolytic cleavage of APP by the α-secretase produces a soluble non-amyloidogenic product $sAPP_\alpha$ with neutrophic effects and promoting synaptogenesis,[14] and an 83 amino acid C-terminal peptide (C83), precluding the production of Aβ. Interestingly, in vivo pharmacological inhibition of T-type Ca^{2+} channels by intraperitoneal injection of NNC-55–0396 (NNC, a specific T-type channel inhibitor[15]) in 14–16 mo old 3xTg-AD mice produced an increase in soluble Aβ ($A\beta_{40}$ and $A\beta_{42}$). However, increased levels of soluble $A\beta_{40}$ and $A\beta_{42}$ were not accompanied by any changes in the number and density of typical senile plaques formed by Aβ. It is possible that the relatively short-term application (2 wk) of NNC precludes the formation of new fibrils. Consistent with this idea, a trend to an increased insoluble $A\beta_{42}$ was noticed, suggesting that long-term treatment of 3xTg-AD mice with T-type channel inhibitors could not only produce an increase in soluble Aβ but may also be accompanied by the formation of new senile plaques. Moreover, active α-secretase ADAM10 and its proteolytic product C83 were found significantly decreased upon in vivo inhibition of T-type Ca^{2+} channels. In contrast, immature ADAM10 was found unchanged, suggesting that blocking T-type Ca^{2+} channels in vivo possibly prevents the maturation of ADAM10 rather than its genic expression. Consistent with in vivo observations, in vitro application of NNC on neuroblaoma cells N2a reduced expression of active ADAM10 and C83 peptide while soluble $A\beta_{40}$ and $A\beta_{42}$ levels were found increased. In contrast, transient overexpression of $Ca_v3.1$ channel in HEK cells stably expressing APP increased the formation of soluble α-APPs suggesting activation of the α-secretase pathway. However, in contrast to in vivo observations, only a non-significant trend to an increased level of active ADAM10 was observed, questioning the molecular mechanisms by which T-type Ca^{2+} channels contribute to the processing of APP. Because of the rather depolarized membrane potential of HEK cells (around -30mV depending on the culture conditions), T-type Ca^{2+} channels are mostly inactivated and their contribution in any Ca^{2+}-dependent processes at rest is rather unlikely. The same applies to neuroblastoma cells. Besides the fact that it remains unclear if T-type Ca^{2+} channels are functionally expressed in N2a cells,[16,17] it is unlikely that undifferentiated cells can generate electrical activity required for T-type Ca^{2+} channel activation. Further investigations will certainly identify the precise molecular mechanisms by which T-type Ca^{2+} channels contribute to the processing of APP. (**Fig. 1**)

In summary, Rice et *al*. documented for the first time an age-dependent decrease in $Ca_v3.1$ T-type Ca^{2+} channel expression, extending the growing list of Ca^{2+}-dependent proteins possibly involved in the aging brain. More importantly, they provided evidence for a functional implication of T-type Ca^{2+} channels in the proteolytic processing of APP where proper channel activity is required for non-amyloidogenic processing of APP, precluding the production of Aβ and possibly associated senile plaques. Interestingly, a recent study documented an increase in insoluble $A\beta_{42}$ production in the aging brain.[18] The age-dependent alteration in $Ca_v3.1$ T-type Ca^{2+} channel expression observed by Rice et al. could certainly contribute to the changes in APP processing with aging and possibly contribute to the pathogenesis of AD. Moreover, considering the critical role of T-type Ca^{2+} channels in supporting physiological functions as learning and memory,[19] it is conceivable that alteration in channel activity could participate in the cognitive declines associated with the aging brain. It thus appears that pharmacological activation of T-type Ca^{2+} channels could represent a promising therapeutic strategy to prevent and/or reverse age-dependent cognitive declines, and possibly to preclude the formation of

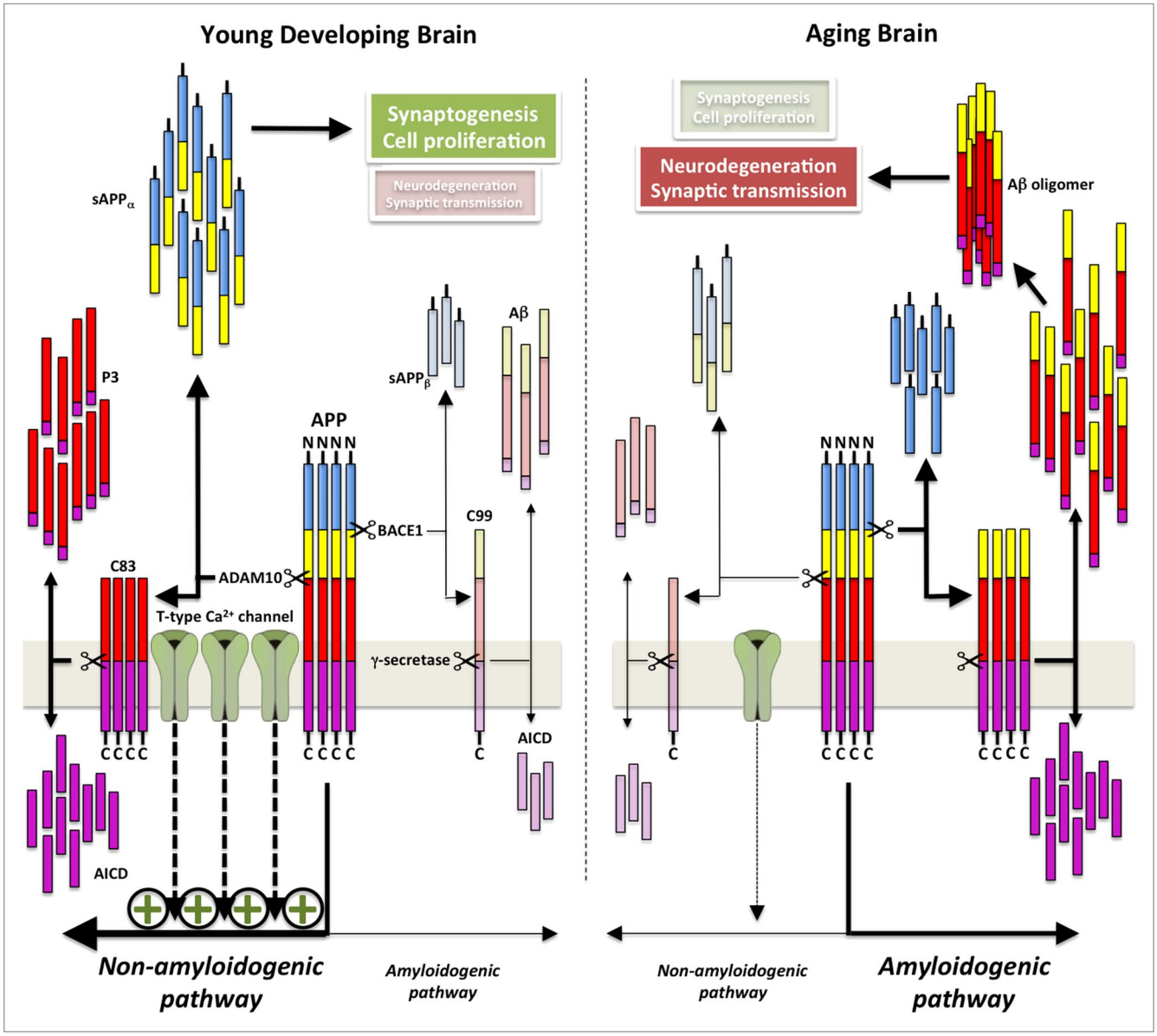

Figure 1. A putative model of functional coupling between T-type Ca^{2+} channels and APP processing. The amyloidogenic pathway is characterized by the proteolytic cleavage of APP by β-secretase (BACE1) and the resulting production of a soluble APP fragment ($sAPP_{\beta}$) and a 99-amino acid C-terminal fragment (C99) that is subsequently processed by γ-secretase to generate neurotoxic Aβ ($A\beta_{40}$ and $A\beta_{42}$) peptides that tend to aggregate and form extracellular senile plaques. In contrast, the non-amyloidogenic processing of APP by α-secretase (ADAM10) produces an 83-amino acid C-terminal fragment (C83) precluding the formation of APP, and a soluble $sAPP_{\alpha}$ product with neurotrophic effects that counteract apoptotic signaling and promote synaptogenesis. In the young developing brain, activation of T-type Ca^{2+} channels stimulates non-amyloidogenic processing of APP, possibly by facilitating the maturation of ADAM10, which supports neuronal growth and synaptogenesis. In contrast, alteration of T-type Ca^{2+} channel expression in the aging brain favors amyloidogenic processing of APP and associated neuronal damages.

Aβ senile plaques that is still considered as one of the main hypothesis of AD.[20,21] Consistent with this idea, it was recently shown that T-type Ca^{2+} channels are the pharmacological targets of the cognitive enhancer ST101.[17] In contrast, pharmacological inhibition of T-type Ca^{2+} channels has recently emerged as a promising therapeutic strategy for the treatment of some forms of epilepsy,[22] as well as for the management of neuropathic pain.[23,24] Hence, if the development of new T-type Ca^{2+} channel modulators is emerging as especially attractive therapeutic strategy for the treatment of various neurological disorders, their general use in clinic has to be cautious to not face the *Jekyll and Hide syndrome* of T-type channels like it has been shown for Aβ.[25]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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