Novel therapeutics from old pharmaceuticals: The Ca\(^{2+}\)/cAMP signaling interaction as a new pharmaceutical target for treatment of diseases related to aging

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Abstract

It is well established that an imbalance of intracellular Ca\(^{2+}\) homeostasis contributes to the disease progression of neurodegenerative illnesses related to aging, such as Alzheimer’s (AD) and Parkinson’s (PD) diseases. Therefore, regulation of intracellular Ca\(^{2+}\) homeostasis may represent a new strategy for treatment of these diseases. Our recent discovery of the involvement of the crosstalk (or interaction) between signalling pathways mediated by the intracellular second messengers Ca\(^{2+}\) and cAMP (Ca\(^{2+}\)/cAMP signaling interaction) in the neurotransmission and neuroprotection has contributed to the understanding of pathophysiology and pharmacology of diseases associated to neurodegeneration because of aging. Interestingly, this discovery emerged from many medical reports performed since 1975 that reported that L-type Ca\(^{2+}\) channel blockers (CCBs) prescribed in antihypertensive pharmacotherapy decreased arterial pressure, but produced typical symptoms of increase of sympathetic activity such as tachycardia and increment of catecholamine plasma levels. Although these off-label effects of CCBs have been initially credited to adjust reflex of arterial pressure, this mysterious phenomenon remained uncertain for forty years. In 2013, we revealed that this increase of sympathetic activity resulted from the increase of transmitter release from sympathetic neurons and adrenal chromaffin cells stimulated by CCBs due to its direct action on the Ca\(^{2+}\)/cAMP signaling interaction. In addition, we discovered that this modulatory action of CCBs increases the intracellular levels of cAMP, attenuating neuronal death caused by cytosolic Ca\(^{2+}\) overload due probably to the activation of cellular survival pathways mediated by cAMP-response element binding protein (CREB). Then, our discovery of the role of the Ca\(^{2+}\)/cAMP signaling interaction in the neurotransmission and neuroprotection may open a large avenue for the progress of a new pharmacological strategy more effective and safer for treating neurodegenerative diseases related to aging (AD and PD).

Keywords: Ca\(^{2+}\)/cAMP signaling interaction; Alzheimer’s disease, Parkinson’s disease.
1. Introduction

Several medical reports have described (since 1970’s) that acute and chronic prescription of L-type Ca\(^{2+}\) channel blockers (CCBs) in patients suffering with hypertension, such as nifedipine and verapamil, decreased arterial pressure but produced typical symptoms of increase of sympathetic activity such as tachycardia and increment of catecholamine plasma levels [1]. Although these off-label effects of CCBs have been initially credited to adjust reflex of arterial pressure, the working principle involved in these CCBs-effects remained uncertain for decades.

Since 1975, some studies performed in isolated tissues highly innervated by sympathetic nervous system (rodent vas deferens), to eliminate the impact of adjusting reflex, reported that responses mediated by these nerves were completely repressed by L-type CCBs in high concentrations (>1 μmol/L), but unexpectedly and puzzlingly potentiated in concentrations below 1 μmol/L, characterizing CCBs-induced sympathetic hyperactivity [2-5]. During almost forty years, this contradictory increase of sympathetic activity produced by L-type CCBs, so-called “calcium paradox”, remained uncertain.

In 2013, we revealed that this paradoxical increase of sympathetic activity produced by L-type CCBs is due to its direct action on the crosstalk (or interaction) between signalling pathways mediated by the intracellular second messengers Ca\(^{2+}\) and cAMP (Ca\(^{2+}\)/cAMP signaling interaction) [5]. Our studies have proposed that pharmacological modulation of the Ca\(^{2+}\)/cAMP signaling interaction by use of the L-type CCBs and compounds that increase the cytosolic concentration of cAMP (cAMP-enhancer compounds) could be effective in enhancing neurotransmission and neuroprotection in neurological and psychiatric disorders resulting from neurotransmission deficit and neuronal death [5-11].

The growing increase in the life expectancy of the world's population has increased the concern about neurodegenerative diseases related to aging, such as Alzheimer's (AD) and Parkinson’s (PD) diseases. In accordance to a 2015 United Nations statement on world population aging, the amount of people aged 60 and older worldwide is predictable to more than double in the next 35 years, reaching almost 2.1 billion people. A considerable amount of this growth will come from developing regions of the world, although the oldest old, who are more than 80 years of age, are the wildest growing segment of the population in developed regions. Despite the enhancements in life expectancy, related neurodegenerative conditions have arguably become the most feared maladies of older people. Then, in this article we will discuss how the pharmacological modulation of the Ca\(^{2+}\)/cAMP signaling interaction could be a new therapeutic approach to treat the neurodegenerative diseases related to aging, especially AD and PD.

2. Role of the Ca\(^{2+}\)/cAMP signaling interaction in neurotransmission

Many experiments studies originated decades ago, using adrenal chromaffin cells as cellular model, established the notion of coupling of stimulus-secretion to explain transmitter release from central and peripheral neurons. In 1970’s, it was discovered that an increase in the cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_{c}\)) constitutes an elementary requirement to trigger release by exocytosis of secretory vesicles containing transmitter (catecholamines, purines and other substances) in adrenal
In 1990’s, it was discovered a direct relationship between rise in $[Ca^{2+}]_c$, and rapid transmitter release from adrenal chromaffin cells [13]. In addition to $Ca^{2+}$, other intracellular messengers are involved in the release of neurotransmitter and hormones. In 1988, it was discovered that elevation of intracellular cAMP concentration ([cAMP]c) mediated by activation of adenylyl cyclases (ACs) with forskolin enhanced exocytosis of secretory vesicles in adrenal chromaffin cells [14]. Although these evidences indicated that both $Ca^{2+}$ and cAMP participate of transmitter exocytosis from neurons, the interaction between $Ca^{2+}$ and cAMP in this response remained uncertain for decades.

In 2013, we discovery that neurotransmitter release from sympathetic neurons is finely regulated by interaction between signalling pathways mediated by the intracellular second messengers $Ca^{2+}$ and cAMP, named $Ca^{2+}$/cAMP signaling interaction [5]. Using isolated tissues highly innervated by sympathetic nerves (rat vas deferens) stimulated by electrical pulses, we demonstrated that responses mediated by these nerves were reduced and completely repressed by L-type CCBs in high concentrations (>1 μmol/L), but puzzlingly increased in concentrations below 1 μmol/L, characterizing CCBs-induced sympathetic hyperactivity [5]. As the activity of ACs is regulated by $Ca^{2+}$, the reduction of $[Ca^{2+}]_c$ produced by L-type CCBs results in increase of activity of ACs and elevation of [cAMP]c [5]. The elevation of [cAMP]c activates cyclic AMP-dependent protein kinase or kinase A (PKA) that activates endoplasmic reticulum (ER) $Ca^{2+}$ channels, such as ER-$Ca^{2+}$ channels regulated by ryanodine receptors (RyR), stimulating $Ca^{2+}$ release [5]. This $Ca^{2+}$ release from ER enhances number of secretory vesicles docked in plasma membrane, increasing neurotransmitter release and synaptic concentration of neurotransmitters [5-11].

Then, we demonstrated that the reduction of $Ca^{2+}$ influx through L-type voltage-activated $Ca^{2+}$ channels (VACC) produced by CCBs increases synaptic transmission due to enhance of neurotransmitter release [5]. Our discovery solved the enigmatic “calcium paradox” of almost forty years involved in increase of sympathetic activity produced by L-type CCBs due to its direct action on the $Ca^{2+}$/cAMP signaling interaction [5-11].

In addition, our studies also showed that combined use of the L-type CCBs and cAMP-enhancer compounds, such as AC activators and phosphodiesterases (PDEs) inhibitors, produced potentiation of sympathetic neurotransmission due to additional increase of neurotransmitter release from sympathetic nerves [5]. We demonstrated that the magnitude of contractile responses mediated by neurotransmitter released from sympathetic nerves by means electrical field stimulation in rat vas deferens (neurogenic contractions) were significantly reduced by L-type CCBs (verapamil) in high concentrations (>1 μmol/L), but puzzlingly increased in concentrations below 1 μmol/L, characterizing CCBs-induced sympathetic hyperactivity (figure 1). This paradoxical increase of neurogenic contractions were significantly potentiated by pre-treatment of vas deferens with cAMP-enhancer compounds, such as AC activators (forskolin) and phosphodiesterase (PDE) inhibitors (rolipram and isobutyl methyl xanthine (IBMX)) (figure 1). These finding indicated that the pharmacological modulation of neural $Ca^{2+}$/cAMP signaling interaction enhances neurotransmitter release causing increase of synaptic transmission [5-11]. Then, the pharmacological modulation of this interaction could be a novel goal to increase neurotransmission in neurodegenerative disease related to aging characterized by severe deficit in central neurotransmission, such as AD and PD.
Figure 1: Increase of neurotransmission produced by pharmacological modulation of neural Ca\(^{2+}\)/cAMP signaling interaction. (A) Records showing that contractile responses mediated by neurotransmitter released from sympathetic nerves by means of electrical field stimulation in rat vas deferens (neurogenic contractions) were significantly reduced by L-type CCBs (verapamil) in high concentrations (>10\(^{-6}\) M), but paradoxically increased in concentrations below 10\(^{-6}\) M, characterizing CCBs-induced sympathetic hyperactivity. This increase of neurogenic contractions by verapamil (<10\(^{-6}\) M) was potentiated by pre-treatment of isolated tissue with cAMP-enhancer compounds, such as rolipram 10\(^{-7}\) M (B), IBMX 10\(^{-6}\) M (C) and forskolin 10\(^{-7}\) M (D). This potentiation by cAMP-enhancer compounds was prevented by inhibition of AC with SQ 22536 (Data not showed). Each point below the record represents molar concentration of verapamil (interval of 0.5 log unity). Each line below the record represents incubation time with cAMP-enhancer compounds. Representative records extracted from [5].

3. Role of the Ca\(^{2+}\)/cAMP signaling interaction in neuroprotection

It is well established that an imbalance of intracellular Ca\(^{2+}\) homeostasis, especially cytosolic Ca\(^{2+}\) overload, contributes to the disease progression of neurodegenerative diseases related to aging, such as AD and PD [5-11]. Therefore, regulation of intracellular Ca\(^{2+}\) homeostasis may represent a new strategy for treatment of these diseases. As previously mentioned, blockade of the L-type VACC by CCBs reduces Ca\(^{2+}\) influx resulting in attenuation of [Ca\(^{2+}\)]\(_{\text{c}}\) and increase of [cAMP]\(_{\text{c}}\) [5-11]. This functional Ca\(^{2+}\)/cAMP signaling interaction regulates various cellular responses, including neurotransmitter release [5-11].
Recently, it was showed that the treatment with L-type CCBs reduces motor symptoms and attenuates progressive neuronal death in animal model of degenerative disease, suggesting that L-type CCBs are potentially viable neuroprotective agents [15, 16]. These finding reinforced the idea that attenuation of cytosolic Ca\(^{2+}\) overload produced by L-type CCBs due to blockade of Ca\(^{2+}\) influx through L-type VACC could be an excellent pharmacological strategy to attenuate or prevent death of neurons in neurodegenerative diseases, such as PD and AD.

In addition, some reports demonstrated that increase of [cAMP]c stimulates neuroprotective response attenuating neuronal death due probably to activation of cellular survival pathways mediated by cAMP/PKA/ cAMP-response element binding protein (CREB)-dependent intracellular signaling pathway [17-20]. In this way, the pharmacological modulation of the Ca\(^{2+}/\text{cAMP}\) signaling interaction by combination of L-type CCBs and cAMP-enhancer compounds could stimulate neuroprotective response due to enhance of [cAMP]c and attenuation of cytosolic Ca\(^{2+}\) overload [5-11]. Thus, pharmacological modulation of this interaction could be a new neuroprotective therapeutic strategy to slow the progression of neurodegenerative diseases related to aging, such as AD and PD. Figure 2 shows how pharmacological modulation of the Ca\(^{2+}/\text{cAMP}\) signaling interaction by combination of L-type CCBs and cAMP-enhancer compounds can produce increase of neurotransmission and neuroprotection.

**Figure 2**: Increase of transmitter release and attenuation of neuronal death (neuroprotection) produced by pharmacological modulation of the Ca\(^{2+}/\text{cAMP}\) signaling interaction by combined use of L-type Ca\(^{2+}\) channel blockers (CCBs) and cAMP-enhancer drugs. PDE - phosphodiesterase inhibitors. Figure extracted from [11].
4. Pharmacological modulation of neural Ca\(^{2+}\)/cAMP signalling interaction as a new therapeutic strategy for treatment of Alzheimer’s disease (AD)

AD is a continuous neurodegenerative disorder related to aging characterized by cognitive and memory deterioration. Neuritic plaques characterize the pathological status of AD, and are respectively related to the accretion of the β-amyloid peptide (Aβ) in brain tissues [21, 22]. According to the amyloid hypothesis, the overproduction of Aβ is a consequence of the disturbance of homeostatic processes that control the proteolytic cleavage of the amyloid precursor protein (APP). Genetic and age-related factors could contribute to a metabolic change, favoring the amyloidogenic processing of APP in detriment of the physiological secretory pathway [21, 22].

The neurotoxic potential of the Aβ results from its biological effects that stimulate aggregation. These progressions, along with a reduction of Aβ clearance from the brain, leads to the extracellular accretion of Aβ, and the subsequent activation of neurotoxic pathways that ultimately lead to cytoskeletal changes, neuronal dysfunction and death of cells [21]. Intracerebral amyloidosis development in AD patients is in an age-dependent manner, but recent indications show that it may be observed in some subjects as early as in the third or fourth decades of life, with increasing extent in late middle age, and highest approximations in old age [21-23].

Therapies whose main goal consist in the modification of amyloid-related cascades may be regarded as promising goals to attenuate or even to prevent dementia [21]. Therefore, the cumulative data on the pathogenesis of AD derived from basic science models will be with any luck be translated into clinical practice in the forthcoming years. Other goals relevant to AD have also been considered in the last years for creating multitarget compounds [24, 25].

In addition to what has been discussed earlier, acetylcholinesterase (AChE) is another important goal for treating the pathogenesis of AD (cholinergic dysfunction hypothesis). Considering the well-accepted hypothesis of accumulation of the Aβ in AD, this relies in the reduction of neurotransmitter acetylcholine (ACh) release in central cholinergic nervous system involved in cognitive function. Thus, the inhibition of ACh degradation by AChE is a potential goal to treat AD [24-26].

An imbalance of intracellular Ca\(^{2+}\) homeostasis also contributes to the pathogenesis of aging-related neurodegenerative diseases, including AD. Several evidences suggest that aging weakens ability of the brain intracellular Ca\(^{2+}\) removal which is likely to stimulate cellular damage due to cytosolic Ca\(^{2+}\) overload leading to neural death and resultant cognitive dysfunction, such as AD [27]. Therefore, regulation of intracellular Ca\(^{2+}\) homeostasis may represent a new strategy for treatment of AD.

A 10-year follow-up study (2000 to 2010), involving 82,107 hypertensive patients of more than 60 years of age, showed that use of L-type CCBs reduced arterial pressure by dilating blood vessels, and risk of dementia in hypertensives, suggesting that these drugs could be clinically used to treat AD [16]. Supportive discoveries for the neuroprotective properties of CCBs have been confirmed in 1,241 elderly hypertensive patients with memory dysfunction [28]. The prescription of CCBs decreased the risk of cognitive impairment and AD not correlated to blood pressure levels when compared to patients not
taking CCBs [28]. The long-term properties of antihypertensive therapy started with a long-acting dihydropinidine (nitrendipine), which has been demonstrated in the double-blind placebo-controlled Syst-Eur trail, in which the incidence of dementia was decreased by 55% [29].

Some studies have proposed that hybrid mixtures having the moieties of tacrine, a potent inhibitor of brain and peripheral AChE, and nimodipine, a L-type CCBs could be useful to treatment of AD [24,25]. In addition, galantamine, a mild AChE inhibitor and an allosteric ligand of nicotinic receptors has been used to progress improvement of cognition and behaviour in patients with AD [26].

It was showed in AD model rats that cAMP-enhancer compounds, such as nobiletin (a polymethoxylated flavone from citrus peels) and oxyntomodulin (a proglucagon-derived peptide that co-activates the GLP-1 receptor and the glucagon receptor), produce neuroprotective effect mediated by intracellular cAMP production, activation of PKA and MAPK pathways and phosphorylation of CREB [18, 20].

Our discovery of the involvement of the Ca\textsuperscript{2+}/cAMP signaling interaction in the neurotransmission and neuroprotection has produced important advances in the understanding of the pathophysiology and pharmacology of AD [5-11]. These advances allowed us to propose that pharmacological modulation of the Ca\textsuperscript{2+}/cAMP signaling interaction produced by combined use of the L-type CCBs (used in the antihypertensive therapy), such as isradipine, and cAMP-enhancer compounds (used in the anti-depressive therapy) such as rolipram, could represent a new therapeutic strategy for treatment of AD in humans.

Pharmacological modulation of the Ca\textsuperscript{2+}/cAMP signaling interaction by combination of the L-type CCBs and cAMP-enhancer drugs could attenuate ACh release deficit, increasing central cholinergic neurotransmission associated to the control of cognitive function. In addition, pharmacological modulation of this interaction could subsidize the reducing of neuronal death due to attenuation of cytosolic Ca\textsuperscript{2+} overload, increase of [cAMP]c and stimulation of cell survival pathways probably mediated by activation of cellular survival pathways regulated by AMP/PKA/CREB-dependent intracellular signaling pathway [17-20]. Thus, pharmacological modulation of Ca\textsuperscript{2+}/cAMP signaling interaction could be a new neuroprotective therapeutic goal to slow the progression of AD [5-11].

5. Pharmacological modulation of neural Ca\textsuperscript{2+}/cAMP signalling interaction as a new therapeutic strategy for treatment of Parkinson’s disease (PD)

PD is progressive neurodegenerative disorder associated to aging that has grown crescent social and economic impact in the western society. While the characteristic motor symptoms of PD have been described for centuries, cognitive impairment has only recently been documented as a central feature. All nowadays therapies are symptomatic and fail to reverse or halt the progression of dopaminergic neurons loss.
Dopamine loss in the substantia nigra, which results from reduction of dopamine release in striatal dopaminergic neurons due to neuronal death, outcomes in the recognizable core signs of asymmetrical bradykinesia and hypokinesia (slowness and reduced amplitude of movement), muscle rigidity (stiffness) and rest tremor, consequences from modifying motor control. Rest tremor, prominent asymmetry and a good response to levodopa are the features that most accurately predict Parkinson pathology [30,31]. The tremor-dominant form of Parkinson tends to course a more benign course than typical PD. Early falls or autonomic symptoms, and a response to Parkinson medicines should raise evidences about the diagnosis [30,31]. Medication-induced parkinsonism due to generally prescribed dopamine-blocking medications, such as antipsychotics (eg, haloperidol, risperidone) and antiemetics (eg, metoclopramide, prochlorperazine) should be excluded in Parkinson’s patients.

Functional imaging of the dopaminergic system using cerebral single photon emission computed tomography or positron emission tomography can be valuable in diagnosis of early Parkinson. Positron emission tomography studies examining the rate of decline in dopamine-producing cells suggest that humans have already lost 50 %–70 % of their nigral neurons, before they advance motor symptoms, and it has been estimated that the duration of this “presymptomatic” phase is about 5 years [30, 31].

Early diagnoses will become a critical problem if effective neuroprotective drugs become accessible. In fact, enhancing dopamine, mainly by Levodopa combined with a dopa-decarboxylase inhibitor remains the most powerful drug therapy for reversing motor impairment. A higher maintenance dose of Levodopa (eg, 200 mg three times daily compared with an initial dose of 100 mg three times daily) provides slightly greater benefit for reducing motor symptoms, but at the cost of earlier wearing-off symptoms and dyskinesias [30,31].

The combined concepts from novel ideas may lead to developments in PD research with the promise of finding compounds that are both effective, and fast-acting, including in patients who have tried other therapies with restricted success. In conclusion, new visions for more efficient pharmacological treatments of PD are clearly needed.

Several evidences suggest that an imbalance of intracellular Ca\(^{2+}\) homeostasis contributes to the pathogenesis of PD, thus regulation of intracellular Ca\(^{2+}\) homeostasis may represent a new strategy for treatment of PD. Several evidences suggest that aging impairs ability of the brain intracellular Ca\(^{2+}\) degradation which is likely to induce cellular damage leading to death of dopaminergic neurons in the substantia nigra and resulting in motor dysfunction due to reduction of dopamine release in striatal dopaminergic neurons.

A phase II clinical trial published in 2016 showed that treatment with is radipine was safely tolerated to reduce motor symptoms by PD patients [32]. In addition, it was showed that the treatment with L-type CCBs such as is radipine reduces motor symptoms and attenuates progressive death of dopamine neurons from substantia nigra in animal model of PD [15]. This study showed that is radipine produces a dose-dependent sparing of dopaminergic fibers and cell bodies at concentrations achievable in humans [15], suggesting that L-type CCBs are potentially viable neuroprotective agents useful for PD treatment. These finding reinforced the idea that attenuation of cytosolic Ca\(^{2+}\) overload produced by L-type CCBs...
due to blockade of Ca\textsuperscript{2+} influx through L-type VACC could be an excellent pharmacological strategy to attenuate or prevent neuronal death in PD.

Our discovery of the involvement of the Ca\textsuperscript{2+}/cAMP signaling interaction in the neurotransmission and neuroprotection has contributed to understanding of the pathophysiology and pharmacology of PD [5-11]. These advances allowed us to propose that pharmacological modulation of the Ca\textsuperscript{2+}/cAMP signaling interaction produced by combined use of the L-type CCBs and cAMP-enhancer compounds could represent a new therapeutic strategy for treatment of PD in humans.

Pharmacological modulation of the Ca\textsuperscript{2+}/cAMP signaling interaction could attenuate dopamine release deficit, increasing central dopaminergic neurotransmission involved in the control of motor function. In addition, pharmacological modulation of this interaction could contribute to reduce death of dopaminergic neurons due to attenuation of cytosolic Ca\textsuperscript{2+} overload, increase of [cAMP]c and stimulation of cell survival pathways probably mediated by activation of cellular survival pathways regulated by AMP/PKA/CREB-dependent intracellular signaling pathway [17-20]. Thus, pharmacological modulation of Ca\textsuperscript{2+}/cAMP signaling interaction could be a new neuroprotective therapeutic strategy to slow the progression of PD [5-11].

6. Conclusion

Our discovery which revealed that Ca\textsuperscript{2+}/cAMP signaling interaction is involved in sympathetic hyperactivity could promote important advances in the pathophysiology and pharmacology of the neurological and psychiatric disorders related to aging. These advances can contribute to drug development more effective and safer to revert clinical symptoms of neurological and psychiatric disorders, such as AD and PD.

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