

Review Article**Recent advances in pharmacotherapy of neurological and psychiatric disorders promoted by discovery of the role of Ca²⁺/cAMP signaling interaction in the neurotransmission and neuroprotection****Leandro Bueno Bergantin, Ph.D. ; Afonso Caricati-Neto, Ph.D.****Laboratory of Autonomic and Cardiovascular Pharmacology,**Department of Pharmacology, Escola Paulista de Medicina, Universidade Federal de São Paulo (UNIFESP), 55 11 5576-4973,**Rua Pedro de Toledo, 669 – Vila Clementino, São Paulo – SP, Brazil, Postal Code: 04039-032.*

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Abstract

Our discovery of the involvement of the interaction between intracellular signalling pathways mediated by Ca²⁺ and cAMP (Ca²⁺/cAMP signaling interaction) in the neurotransmission and neuroprotection has produced important advances in the understanding of the pathophysiology and pharmacology of neurological and psychiatric disorders, such as Alzheimer's and Parkinson's diseases. Interestingly, this discovery initiated decades ago when numerous clinical studies have reported that L-type Ca²⁺ channel blockers (CCBs) used in antihypertensive pharmacotherapy decreased arterial pressure, but produced typical symptoms of sympathetic hyperactivity such as tachycardia and increment of catecholamine plasma levels. Despite these adverse effects of CCBs have been initially attributed to adjust reflex of arterial pressure, during almost four decades this enigmatic phenomenon named "calcium paradox" remained unclear. In 2013, we discovered that these "calcium paradox" results of transmitter release from sympathetic neurons and adrenal chromaffin cells stimulated by CCBs due to its modulatory action on the Ca²⁺/cAMP signaling interaction. In addition, we discovered that this modulatory action attenuates neuronal death triggered by cytosolic Ca²⁺ overload. These findings open a large avenue for the development of new pharmacological strategies more effective for the treatment of neurological and psychiatric disorders resulting of neurotransmitter release deficit, and neuronal death.

Keywords: Ca²⁺/cAMP signaling interaction; neurotransmission; neuroprotection; neurological/psychiatric disorders

Introduction

Since 1970's, several clinical studies have reported that acute and chronic administration of L-type Ca²⁺ channel blockers (CCBs) in hypertensive patients, such as nifedipine and verapamil, decreased arterial pressure but produced typical symptoms of sympathetic hyperactivity such as tachycardia and increment of catecholamine plasma levels (Grossman et al., 1998). Despite these adverse effects of CCBs have been initially credited to adjust reflex of arterial pressure, the cellular and

molecular mechanisms involved in this CCBs-effects remained unclear for decades. Our previous studies performed in isolated tissues richly innervated by sympathetic nerves (rat vas deferens) to exclude the influence of adjusting reflex, showed that neurogenic responses were completely inhibited by L-type CCBs in high concentrations (>1 µmol/L), but unexpectedly and paradoxically potentiated in concentrations below 1 µmol/L, characterizing CCBs-induced sympathetic hyperactivity (Kreye et al., 1975; French et al., 1981; Moritoki et al., 1987). During almost four decades, these paradoxical effects of CCBs named by us as "calcium paradox" remained unclear.

In 2013, we discovered that this paradoxical sympathetic hyperactivity produced by L-type CCBs is due to its modulatory action on the interaction between the intracellular signaling pathways mediated by Ca²⁺ and cAMP (Ca²⁺/cAMP signaling interaction). Our studies have

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showed that pharmacological modulation of the $\text{Ca}^{2+}/\text{cAMP}$ signaling interaction by use of the L-type CCBs and compounds which increase cytosolic cAMP concentration ($[\text{cAMP}]_c$) named cAMP-enhancer drugs, could be useful to increase neurotransmission and neuroprotection in neurological and psychiatric disorders, such as Parkinson's and Alzheimer's diseases (Bergantin et al., 2013; Caricati-Neto et al., 2015; Bergantin et al., 2015; Bergantin et al., 2016).

Role of the $\text{Ca}^{2+}/\text{cAMP}$ signaling interaction in neurotransmission

Many experiments studies initiated decades ago, using adrenal chromaffin cells as cellular model, established the notion of stimulus-secretion coupling to explain transmitter release from central and peripheral neurons. In 1970's was discovered that a rise in the cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_c$) is an elementary requirement to trigger transmitter release from adrenal chromaffin cells (Baker et al., 1978). In 1990's was showed a direct relationship between rise in $[\text{Ca}^{2+}]_c$ and rapid transmitter release from adrenal chromaffin cells (Neher et al., 1993). It was also showed that increase of $[\text{cAMP}]_c$ in adrenal chromaffin cells due to activation adenylate cyclase by forskolin enhances release of secretory vesicles containing transmitters (catecholamines, purines and other substances) (Chern et al., 1988). These findings support that both Ca^{2+} and cAMP are involved in the regulation of neurotransmitter release at many peripheral and central synapses of mammals, including sympathetic synapses.

In 2013, we discovery that neurotransmitter release from sympathetic neurons is finely regulated by interaction between intracellular signalling pathways mediated by Ca^{2+} and cAMP, named $\text{Ca}^{2+}/\text{cAMP}$ signaling interaction (Bergantin et al., 2013). In fact, the hypothesis for a suitable $\text{Ca}^{2+}/\text{cAMP}$ signaling interaction has been widely studied in different cell types and tissues. This interaction results in synergistic actions of these intracellular messengers on cell functions regulated by adenylyl cyclases (ACs), or phosphodiesterases (PDEs) (Bergantin et al., 2013; Caricati-Neto et al., 2015; Bergantin et al., 2016). The $\text{Ca}^{2+}/\text{cAMP}$ signaling interaction has particularly been extensively studied at the endoplasmic reticulum (ER) Ca^{2+} channels, such as ER- Ca^{2+} channels regulated by ryanodine receptors (RyR) (Bergantin et al., 2013; Caricati-Neto et al., 2015; Bergantin et al., 2016). Our studies established that $\text{Ca}^{2+}/\text{cAMP}$ signaling interaction plays an important role in neurotransmitter release regulation in neurons and neuroendocrine cells (Bergantin et al., 2013; Caricati-Neto et al., 2015; Bergantin et al., 2015; Bergantin et al., 2016). Thus, pharmacological modulation of this interaction produced by L-type CCBs and cAMP-enhancer drugs could be useful to treat neurological and psychiatric disorders resulting of neurotransmitter release deficit, such as Parkinson's and

Alzheimer's diseases.

Role of the $\text{Ca}^{2+}/\text{cAMP}$ signaling interaction in neuroprotection

It is well established that cytosolic Ca^{2+} overload is directly involved in neuronal death in various neurodegenerative diseases, including Alzheimer's and Parkinson's diseases (Bergantin et al., 2013; Caricati-Neto et al., 2015; Bergantin et al., 2015; Bergantin et al., 2016). Recently was showed that the treatment with L-type CCBs such as isradipine reduces motor symptoms and attenuates progressive death of dopamine neurons from substantia nigra in animal model of Parkinson's disease (Ilijic et al., 2011). It was showed that isradipine produces a dose-dependent sparing of dopaminergic fibers and cell bodies at concentrations achievable in humans (Ilijic et al., 2011), suggesting that L-type CCBs are potentially viable neuroprotective agents for Parkinson's disease. A phase II clinical trial published in 2016 showed that treatment with isradipine was safely tolerated to reduce motor symptoms by patients with Parkinson's disease (Swart et al., 2016). In addition, 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 blood pressure and risk of dementia in hypertensives, suggesting that these drugs could be clinically used to treat Alzheimer's diseases (Wu et al., 2016). 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 voltage-activated Ca^{2+} channels (VACC) could be an excellent pharmacological strategy to attenuate or prevent neuronal death in neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases.

As previously mentioned, blockade of the L-type VACC by CCBs reduces Ca^{2+} influx and $[\text{Ca}^{2+}]_c$, increasing ACs activity and $[\text{cAMP}]_c$ (Bergantin et al., 2013; Caricati-Neto et al., 2015; Bergantin et al., 2016). This functional $\text{Ca}^{2+}/\text{cAMP}$ signaling interaction regulates various cellular responses, including neurotransmitter release (Bergantin et al., 2013; Caricati-Neto et al., 2015; Bergantin et al., 2016). Many studies showed that increase of $[\text{cAMP}]_c$ stimulates neuroprotective response attenuating neuronal death due probably to activation of cellular survival pathways mediated by cAMP-response element binding protein (CREB) (Sommer et al., 1995; Xiao et al., 2011; Li et al., 2016). In this way, L-type CCBs and cAMP-enhancer drugs modulates $\text{Ca}^{2+}/\text{cAMP}$ signaling interaction stimulating neuroprotective response due to increase of $[\text{cAMP}]_c$ and attenuation of cytosolic Ca^{2+} overload (Bergantin et al., 2013; Caricati-Neto et al., 2015; Bergantin et al., 2016). Thus, pharmacological modulation of $\text{Ca}^{2+}/\text{cAMP}$ signaling

interaction could be a new neuroprotective therapeutic strategy to slow the progression of neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases.

Pharmacological modulation of Ca²⁺/cAMP signaling interaction: a new avenue for the drug development for the treatment of neurological and psychiatric disorders

Our discovery of the involvement of the Ca²⁺/cAMP signaling interaction in the neurotransmission and neuroprotection has produced important advances in the understanding of the pathophysiology and pharmacology of neurological and psychiatric disorders (Bergantin et al., 2013; Caricati-Neto et al., 2015; Bergantin et al., 2015; Bergantin et al., 2016). These advances allowed us to propose that pharmacological modulation of the Ca²⁺/cAMP signaling interaction produced by combination of the L-type CCBs used in the antihypertensive therapy such as isradipine, and cAMP-enhancer drugs used in the anti-depressive therapy such as rolipram, could represent a new

therapeutic strategy for enhancing neurotransmission and producing neuroprotection in the neurodegenerative diseases, such as Alzheimer disease.

Our studies suggest that combined use of the L-type CCBs and cAMP-enhancer drugs induces enhance of neurotransmission due to increase of neurotransmitter mediated by Ca²⁺ release from ER stimulated by cAMP (Bergantin et al., 2013; Caricati-Neto et al., 2015; Bergantin et al., 2016). This Ca²⁺ release from ER produces increase number of secretory vesicles docked in plasma membrane, increasing neurotransmitter release (Bergantin et al., 2013; Caricati-Neto et al., 2015; Bergantin et al., 2016). Pharmacological modulation of the Ca²⁺/cAMP signaling interaction could be a new therapeutic strategy to treat neurological and psychiatric disorders resulting of neurotransmitter release deficit. In addition, pharmacological modulates of Ca²⁺/cAMP signaling interaction by combined use of the L-type CCBs and

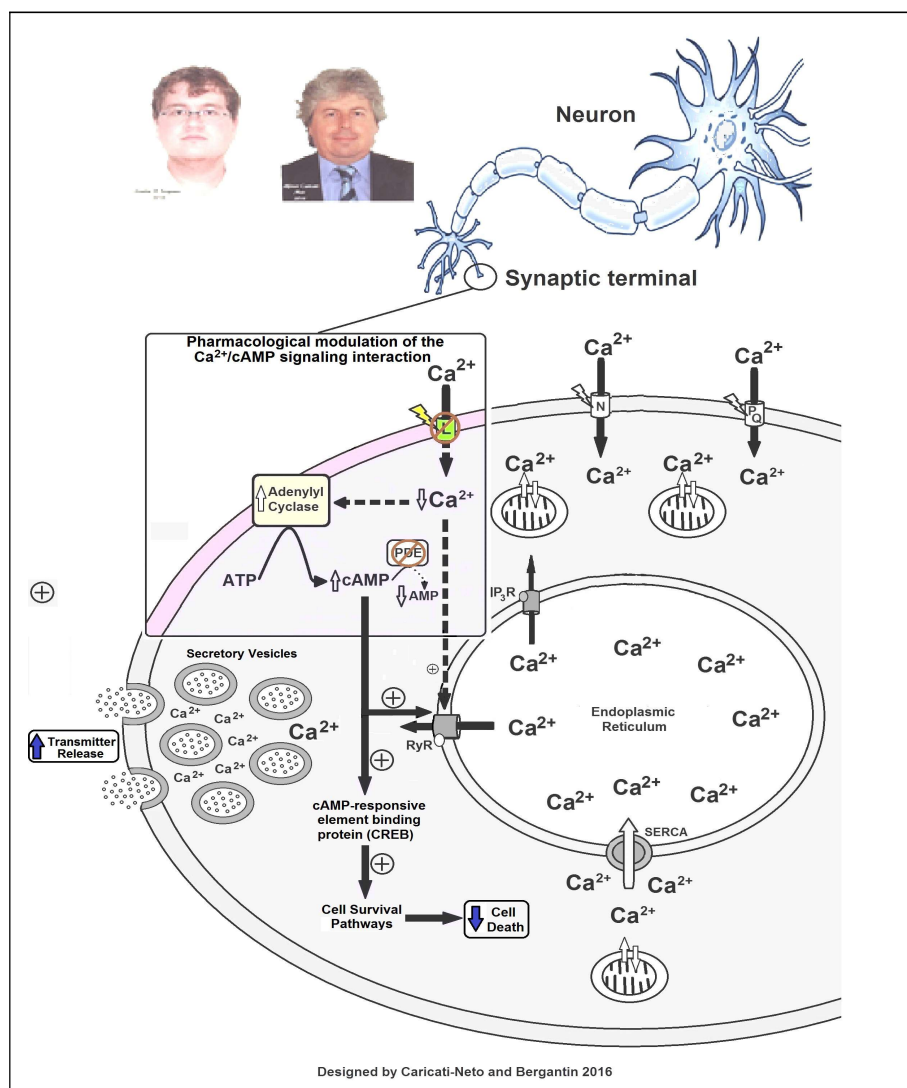


Figure 1. Increase of neurotransmitter release and attenuation of neuronal death produced by pharmacological modulation of the Ca²⁺/cAMP signaling interaction using L-type Ca²⁺ channel blockers (CCBs) and cAMP-enhancer drugs, such as phosphodiesterase (PDE) inhibitors.

cAMP-enhancer drugs leads to reduction of neuronal death due to attenuation of cytosolic Ca^{2+} overload, increase of [cAMP]c and stimulation of cell survival pathways mediated by CREB (Sommer et al., 1995; Xiao et al., 2011; Li et al., 2016). Thus, pharmacological modulation of Ca^{2+} /cAMP signaling interaction could be a new neuroprotective therapeutic strategy to slow the progression of neurodegenerative diseases (Bergantin et al., 2013; Caricati-Neto et al., 2015; Bergantin et al., 2015; Bergantin et al., 2016). Our proposal could open a new avenue for the drug development more effective and safer to reduce clinical symptoms of neurological and psychiatric disorders resulting of neurotransmitter release deficit, and neuronal death triggered by cytosolic Ca^{2+} overload, such as Alzheimer's and Parkinson's diseases. Figure 1 shows how pharmacological modulation of the Ca^{2+} /cAMP signaling interaction using L-type CCBs and cAMP-enhancer drugs can produce increase of neurotransmission and neuroprotection.

Conclusion

Our recent discovery of the Ca^{2+} /cAMP signaling interaction could promote important advances in the pathophysiology and pharmacology of the neurological and psychiatric disorders. These advances can contribute to drug development more effective and safer to prevent clinical symptoms of neurological and psychiatric disorders, such as Alzheimer's and Parkinson's diseases.

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