

# The passion of a scientific discovery: the “calcium paradox” due to $\text{Ca}^{2+}$ /cAMP interaction

Afonso Caricati-Neto and Leandro Bueno Bergantini\*

Department of Pharmacology - Universidade Federal de São Paulo - Escola Paulista de Medicina, Brazil

## Abstract

About 4 years ago, we showed that the paradoxical effects (sympathetic hyperactivity) induced by L-type voltage-activated  $\text{Ca}^{2+}$  channels (VACC) blockers, named by us “calcium paradox” phenomenon, were potentiated by drugs which increase cytosolic cAMP concentration ([cAMP]c-enhancers), for example rolipram, IBMX and forskolin, indicating that the sympathetic hyperactivity induced by VACC blockers is due to interaction of the  $\text{Ca}^{2+}$ /cAMP intracellular signaling pathways ( $\text{Ca}^{2+}$ /cAMP interaction). Then, the pharmacological handling of this interaction produced by combined use of the L-type VACC blockers prescribed in the antihypertensive therapy, and [cAMP]c-accumulating compounds prescribed in the antidepressive therapy, could represent a potential cardiovascular deleterious effect for hypertensive patients due to stimulation of sympathetic hyperactivity. Then, we discussed the role of  $\text{Ca}^{2+}$ /cAMP interaction for neurodegenerative diseases pharmacotherapy. In conclusion, this interaction could be a novel therapeutic target for drug development.

## Introduction

Since 1970's, many medical studies have described that prescription of  $\text{Ca}^{2+}$  channels blockers (CCBs) for hypertensive patients, such as verapamil and nifedipine, produces reduction in peripheral vascular resistance and arterial pressure due to vasodilation, resultant of reduction of  $\text{Ca}^{2+}$  influx through plasmalemal L-type voltage-activated  $\text{Ca}^{2+}$  channels (VACC) in smooth muscle cells, but also produces increase in plasma catecholamines levels and heart rate, typical symptoms of sympathetic hyperactivity [1]. Despite the risks of anti-hypertensive therapy, these adverse effects of L-type VACC blockers were initially attributed to autonomic adjust reflex of arterial pressure. However, several experimental studies performed in 1970's, by studying the effects of L-type VACC blockers in smooth muscle richly innervated by sympathetic neurons (rodent vas deferens) submitted to electrical field stimulation (EFS), demonstrated that these blockers could produce dual effect on sympathetic neurotransmission. In high concentrations ( $>1\mu\text{M}$ ), L-type VACC blockers completely inhibited sympathetic EFS-contractions due to blockade of  $\text{Ca}^{2+}$  influx in smooth muscle cells, but paradoxically increased these contractions in low concentrations ( $<1\mu\text{M}$ ), suggesting that other working principle than only autonomic adjusting reflex is involved in this paradoxical event. However, the fundamental mechanisms involved in this paradoxical effect of L-type VACC blockers remained unclear during almost forty years [2-5].

## Theoretical model of $\text{Ca}^{2+}$ /cAMP interaction

In a study published in 2013 in Cell Calcium, we demonstrated that the paradoxical sympathetic hyperactivity produced by L-type VACC blockers, named by us as “calcium paradox”, is resultant of interference produced by these blockers on the signaling pathways interaction mediated by  $\text{Ca}^{2+}$  and cAMP ( $\text{Ca}^{2+}$ /cAMP interaction) [6]. We showed that simultaneous administration of L-type VACC blockers (verapamil) with drugs which produce increase of cytosolic cAMP concentration ([cAMP]c-enhancers), such as rolipram, IBMX and forskolin, enhanced sympathetically-mediated contractions of vas

deferens [6]. In addition, this enhancement of contraction was reduced by decreasing [cAMP]c caused by adenylyl cyclase (AC) inhibition (SQ 22536), or diminution of  $\text{Ca}^{2+}$  storage from endoplasmic reticulum (ER) by thapsigargin, revealing the involvement of  $\text{Ca}^{2+}$  mobilization from ER in this response [6].

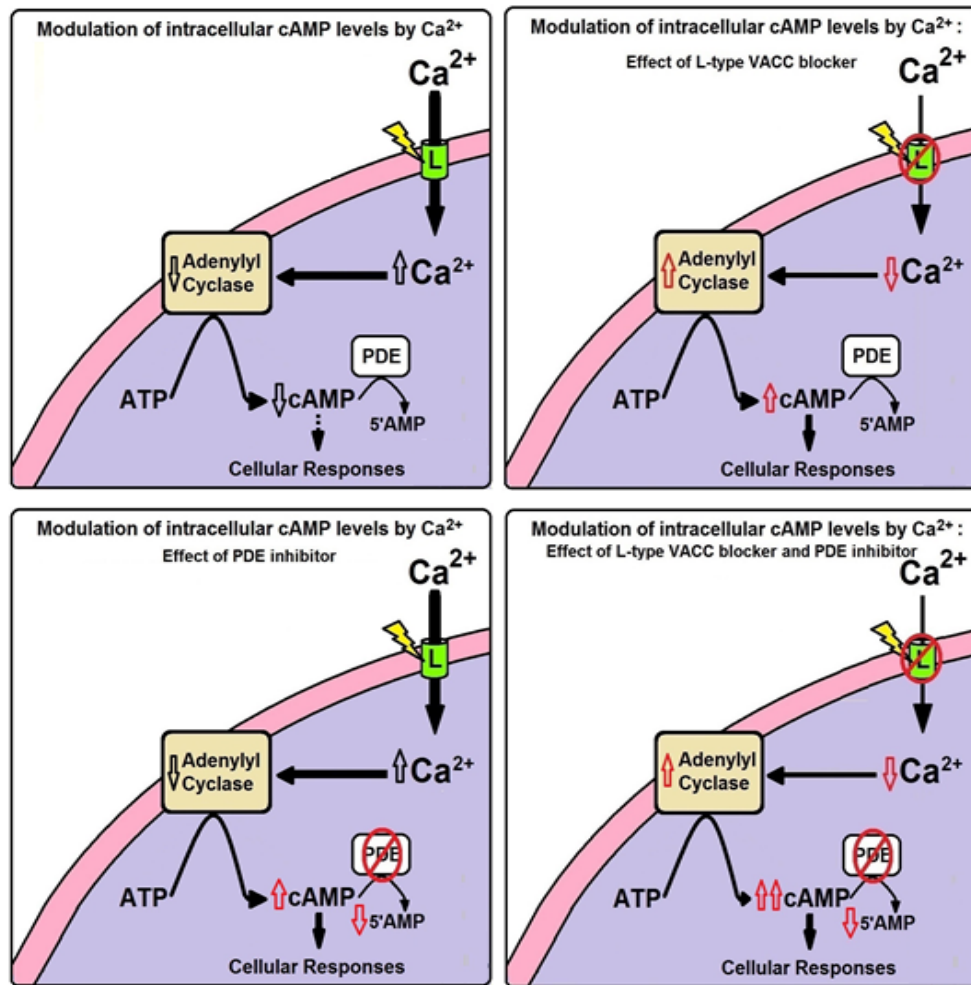
Our studies showed that paradoxical increase of sympathetic activity produced by CCBs, used in anti-hypertensive therapy, results from increment of neurotransmitters release from secretory response of postganglionic sympathetic neurons due to its interference on the  $\text{Ca}^{2+}$ /cAMP interaction [6-8]. Thus, reduction of  $\text{Ca}^{2+}$  influx in these cells by verapamil and other CCBs (in low concentration) increases AC activity, and [cAMP]c, due to decrease of [ $\text{Ca}^{2+}$ ]c [6-8]. It was showed that 5 and 6 isoforms of AC are involved in this AC-inhibition by  $\text{Ca}^{2+}$ . The [cAMP]c increment causes increase of cAMP-stimulated  $\text{Ca}^{2+}$ -release from ER, that in turn, stimulates exocytotic process due to enhance of secretory vesicles recruitment, and docking, to the plasma membrane, priming of fusion machinery, and fusion of vesicles with the plasma membrane [6-8].

Theoretical model of  $\text{Ca}^{2+}$ /cAMP interaction in secretory cells proposed by Bergantini et al. (2013) and Caricati-Neto et al. (2015) illustrates how secretory responses of the neurons and neuroendocrine cells can be modified by pharmacological modulation of intracellular levels of  $\text{Ca}^{2+}$  and/or cAMP (Figure 1). This proposition is reinforcing

**Correspondence to:** Department of Pharmacology - Universidade Federal de São Paulo - Escola Paulista de Medicina, Laboratory of Autonomic and Cardiovascular Pharmacology – 55 11 5576-4973, Rua Pedro de Toledo, 669 – Vila Clementino, São Paulo – SP, Brazil, CEP: 04039-032, E-mail: leanbio39@yahoo.com.br

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**Figure 1.** The Ca<sup>2+</sup>/cAMP interaction in neurons, and neuroendocrine cells, proposed by Bergantin, et al. (2013) [6] and Caricati-Neto, et al. (2015) [8]. This model illustrates how cellular responses of cells can be modified by pharmacological modulation of intracellular levels of Ca<sup>2+</sup> and/or cAMP.

the hypothesis that Ca<sup>2+</sup>/cAMP interaction plays an important role in the sympathetic activity, by regulating secretory response of sympathetic neurons, and adrenal chromaffin cells [6]. In addition, these pharmacological manipulations of the Ca<sup>2+</sup>/cAMP interaction could have important implications for antihypertensive pharmacotherapy [7,8].

### Implications of Ca<sup>2+</sup>/cAMP interaction for neurodegenerative diseases pharmacotherapy: new avenues for drug development

Sympathetic hyperactivity such as tachycardia, and increment of catecholamine plasma levels, have been evidenced by several medical studies dealing with CCBs [1,9-11]. Despite these adverse effects of CCBs have been initially attributed to adjust reflex of arterial pressure, during almost four decades the cellular and molecular mechanisms involved this enigmatic phenomenon named "calcium paradox" remained unclear.

In 2013, we revealed that "calcium paradox" phenomenon resulted from the increment of transmitter release from sympathetic neurons, and adrenal chromaffin cells, stimulated by CCBs due to its interference on the Ca<sup>2+</sup>/cAMP interaction [6]. For excluding the influence of adjusting reflex, we used isolated tissues richly innervated

by sympathetic nerves (rat vas deferens), and we showed that neurogenic contractions of the vas deferens were completely inhibited by L-type CCBs in high concentrations (>1 μmol/L), but unpredictably, and paradoxically, potentiated in concentrations below 1 μmol/L, characterized by sympathetic hyperactivity induced by CCBs [2-5]. The increment of neurotransmitter release from sympathetic neurons due to its pharmacological modulation on the Ca<sup>2+</sup>/cAMP interaction is the cause of this paradoxical sympathetic hyperactivity [5-8] (Figure 1).

Undeniably, several studies showed that neuroprotective response can be achieved by increase of cytosolic cAMP concentration ([cAMP]<sub>c</sub>) [12-19]. In this way, we could propose that a rise of [cAMP]<sub>c</sub> by interfering in the Ca<sup>2+</sup>/cAMP interaction could attenuate neuronal death triggered by cytosolic Ca<sup>2+</sup> overload [12-19]. In conclusion, the pharmacological interference of the Ca<sup>2+</sup>/cAMP interaction produced by combined prescription of the L-type CCBs used in the antihypertensive therapy, and [cAMP]<sub>c</sub> enhancer compounds used in the anti-depressive therapy such as rolipram, could be a new pharmacological strategy for increasing neurotransmission in neurological and psychiatric disorders resulting from neurotransmitter release deficit, and/or neuronal death. These results could open a new path for the drug development more effective and safer for the treatment of neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases.

## Conclusion

The passion of a scientific discovery: new insights for the neuroscience field from the discovery of the “calcium paradox” due to Ca<sup>2+</sup>/cAMP interaction have been emerging to treat neurodegenerative diseases. Pharmacological manipulation of this interaction could be a more effective therapeutic strategy for inducing neurotransmission compromised by neurotransmitter release deficit, and diminishing neuronal death in the neurodegenerative diseases. These results could open a new path for the drug development more effective and safer for the treatment of neurodegenerative diseases, such as Alzheimer’s and Parkinson’s diseases.

## Disclosure statement

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## Conflict of interest statement

The authors declare no conflict of interest.

## Authorship contribution statement

Not applicable to reviews.

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