The “Calcium Paradox” Discovery: Impact in Translational Research

Bergantin LB* and Caricati-Neto A
Department of Pharmacology, Universidade Federal de São Paulo, Brazil

*Corresponding author: Bergantin LB, Department of Pharmacology – Universidade Federal de São Paulo – Escola Paulista de Medicina, Brazil, Tel: 04039-032; E-mail: leanbio39@yahoo.com.br

Abstract
An emerging word in biomedical research nowadays is the “translational research”. Basically, “translating” current knowledge from basic science to approaches for treating human disease - from bench to bedside - is the main concept. Our discovery entitled “calcium paradox” fits properly in this main concept. The enigma of the “calcium paradox” initiated with experiments performed in a classical study model of the neurotransmission (rodent vas deferens). Using this model, some studies performed since 1975 reported that reduction of Ca\(^{2+}\) entry by low concentrations of Ca\(^{2+}\) channel blockers (CCBs: verapamil, diltiazem or nifedipine) produced a paradoxical increase of the contractions mediated by sympathetic nerves, a phenomenon entitled as “calcium paradox”. Recent studies using adrenal chromaffin cells have also demonstrated that nifedipine caused a paradoxical increase of the catecholamine release. Because these compounds are blocking the L-type voltage-activated Ca\(^{2+}\) channels (VACCs), augmented nerve-mediated response due to increase of neurotransmitter release was an unexpected outcome. In 2013, we revealed that the Ca\(^{2+}/\text{cAMP}\) signalling interaction could properly explain the so-called “calcium paradox”. The original paper published by us in Cell Calcium (2013) has appeared four times in ScienceDirect TOP 25 Hottest Articles lists. In conclusion, these findings may dramatically impact in hypertension, neurological/psychiatric diseases, cancer and many other diseases, stimulating the development of new drugs for the pharmacotherapy of these diseases.

Keywords: Calcium Paradox; cAMP; Ca\(^{2+}\); Translational Research

Introduction
Nowadays, an emerging concept in biomedical research is the “translational research”. Basically, “translating” current knowledge from basic science to approaches for treating human disease - from bench to bedside - is the main concept. Our discovery entitled “calcium paradox” fits properly in this concept.

From basic science, we know that in mammals, increases of the concentration of free Ca\(^{2+}\) ions in the cytosol ([Ca\(^{2+}\)\(_{cyt}\)]\(_{i}\)) serve as a messenger signal to couple the stimulus to muscle contraction or to neurosecretion, among other innumerable physiological responses [1,2]. A huge number of experiments performed since the discovery of the role of Ca\(^{2+}\) in the control of the heart beat [3] have set the dogma that in excitable cells, the enhanced Ca\(^{2+}\) entry through voltage-activated Ca\(^{2+}\) channels (VACCs) elicited by depolarising stimuli, trigger muscle contraction and the release of neurotransmitters...
and hormones. Conversely, the mitigation of Ca\textsuperscript{2+} entry produced by VACCs blockers causes a diminution of those responses [4,5].

The above concepts imply that enhanced Ca\textsuperscript{2+} entry during cell depolarisation and/or enhanced Ca\textsuperscript{2+} release from the sarco-endoplasmic reticulum (ER) augments the [Ca\textsuperscript{2+}], and the triggering of the contraction, or secretion responses. However, near four decades ago, a study showed that verapamil at low concentrations produced a paradoxical increase of the rat vas deferens contractions mediated by sympathetic neurotransmission [6]. On the other hand, nifedipine was recently found to paradoxically augment the exocytosis of catecholamine triggered by double-pulse depolarisations from voltage-clamped bovine adrenal chromaffin cells, another interesting model to study sympathetic neurotransmission [7]. How these two blockers of the L-type of VACCs can enhance, instead of reducing, the Ca\textsuperscript{2+}-dependent responses of contraction, and secretion? We properly gave a response to this "calcium paradox" in 2013 through the Ca\textsuperscript{2+}/cAMP signaling interaction (Ca\textsuperscript{2+}/cAMP interaction) [8].

The "Calcium Paradox" Discovery and its Impact in Translational Research

In the vas deferens, both release and postsynaptic actions of noradrenaline (NA) and ATP depend on Ca\textsuperscript{2+} entry through VACCs, and the ensuing elevations of [Ca\textsuperscript{2+}]. [9]. Hence, some authors found that verapamil abolished the electrically-evoked neurogenic contractions of the vas deferens [10,11]. In an earlier study, however, it was reported that verapamil blocked the rat vas deferens contractions sympathetically-mediated, as expected; nevertheless, this study also described that the lower concentrations of verapamil produced a surprising augmentation of those contractions [6]. This paradoxical effect was corroborated in 1981 by French and Scott [12], also in the rat vas deferens contractions induced by nerve-stimulation. Furthermore, six years later a third study reported that verapamil and diltiazem increased the twitch response of the rat vas deferens contractions induced by nerve-stimulation; this result was attributed to an agonist effect of verapamil on L-type VACCs, thus increasing Ca\textsuperscript{2+} entry and neurotransmitter release [13]. Two years later, another study appeared revealing that both, L-type VACCs blockers and activator BAY K 8644, elicited similar augmentations of the neurogenic contractions of the vas deferens; the authors did not provide an explanation for such paradoxical observation [14].

In a study from our laboratory, we could replicate those previous observations in the neurogenically-induced contractions of the rat vas deferens: at lower concentrations verapamil produced a tiny increase, while at higher concentrations the VACCs blocker caused full inhibition of the vas deferens contractions [8]. The exciting finding was that as the high verapamil concentrations, various cAMP enhancers such as phosphodiesterase (PDE) inhibitors rolipram and IBMX (isobutyl methyl xanthine), and adenylyl cyclase (AC) activator forskolin, reduced the neurogenic vas deferens contractions; however, in the presence of cAMP enhancers, the lower concentrations of verapamil caused a severe increase of the neurogenic contractions. The inhibition of AC by SQ 22536 decreased the enhanced contractions, indicating that a Ca\textsuperscript{2+}/cAMP interaction could properly explain the paradoxical results of combined verapamil plus cAMP enhancers, and also the so-called "calcium paradox" [8]. Thus, these findings can dramatically impact the hypertension, neurological/psychiatric disorders, cancer and other diseases [15,16].

Based on these findings, we have anticipated that the pharmacological regulation of the Ca\textsuperscript{2+}/cAMP interaction by combined use of the L-type VACCs blockers and [cAMP]-enhancer compounds could be a novel therapeutic goal for increasing neurotransmission in neurological, and psychiatric disorders, resulted from neurotransmitter release deficit, and neuronal death [15,16]. This pharmacological strategy opens a novel pathway for the drug development more efficient for the treatment of Alzheimer’s and other neurodegenerative diseases [15,16]. In fact, it was demonstrated that the prescription of L-type CCBs reduces motor symptoms, and reduces progressive neuronal death in animal model of neurodegenerative disease, indicating that L-type CCBs are potentially viable neuroprotective pharmaceuticals [17]. Intriguingly, a 1-decade study involving thousands senile hypertensive patients demonstrated that prescription of L-type CCBs reduced blood pressure, and risk of dementia, in hypertensive patients, indicating that these pharmaceuticals could be clinically used to treat neurodegenerative diseases [18]. These results for the neuroprotective effects of CCBs have been reinvestigated in thousands elderly hypertensive patients with memory dysfunction [19]. These studies concluded that patients who have taken CCBs had their risk of cognitive dysfunction decreased, such as Alzheimer’s disease [19]. These findings reinforce the idea that reduction of cytosolic Ca\textsuperscript{2+} overload produced by L-type CCBs due to blockade of Ca\textsuperscript{2+} influx could be an alternative
pharmacological goal to reduce, or prevent, neuronal death in neurodegenerative diseases.

In addition, it has been shown that the dysregulation of intracellular signaling pathways mediated by Ca\(^{2+}\) and cAMP participates in the cancer initiation, tumor formation, tumor progression, metastasis, invasion and angiogenesis. Thereby, proteins involved in these pathways, such as Ca\(^{2+}\) channels and cAMP-dependent protein kinase (PKA), represent potential drug targets for cancer therapy [20]. With this concept in mind, some studies showed that drugs able to interfere with the intracellular Ca\(^{2+}\) signaling such as selective VACCs blockers, as amlodipine, inhibit proliferative response in different cancer cells. In addition, drugs able to increase the intracellular cAMP levels (cAMP-enhancer compounds), such as PDE 4 inhibitors, have been proposed as potential adjuvant, chemotherapeutic or chemopreventive agents in some cancer types, including hepatocellular carcinoma [20]. Then, the pharmacological modulation of the intracellular signaling mediated by Ca\(^{2+}\) and cAMP in the cancer cells may represent a new therapeutic strategy for cancer progression.

In conclusion, the “calcium paradox” may dramatically impact in translational research, stimulating the development of new drugs for the pharmacotherapy of hypertension, neurological/psychiatric diseases, cancer and many other diseases.

**Acknowledgments**

Caricati-Neto and Bergantin thank the continued financial support from CAPES, CNPq and FAPESP (Bergantin’s Postdoctoral Fellowship FAPESP #2014/10274-3).

**References**


