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Clinical Research: Good News Coming from Ca²⁺/cAMP Signaling Interaction

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Abstract

Clinical research plays an important role in medical sciences by determining the safety and efficiency of medications, devices and diagnostic products for human use. Our discovery of the interactions between intracellular signaling pathways as mediated by Ca²⁺ and cAMP (Ca²⁺/cAMP signaling interaction) in neurotransmission could help improve clinical research. This discovery emerged from classical neurotransmission studies using rodent vas deferens as a model. In addition, the concept of Ca²⁺-dependent muscle contraction (Ca²⁺ influx triggers muscle contraction) is amply accepted. Thus, Ca²⁺ channel blockers (CCB) should reduce muscle contraction. Nonetheless, using this model, some studies performed since 1975 reported that reduction of Ca²⁺ influx by low concentrations of CCB (verapamil, diltiazem or nifedipine) produced a paradoxical increase of the contractions mediated sympathetic nerves, a phenomenon known as “calcium paradox”. Recent studies using adrenal chromaffin cells have also demonstrated that CCB caused a paradoxical increase of the catecholamine release. Because these compounds are blocking the L-type voltage-activated Ca²⁺ channels (VACC), an augmented nerve-mediated response due to increased neurotransmitter release was an unexpected outcome. In 2013, we revealed that the Ca²⁺/cAMP signaling 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 significantly impact on cardiovascular and neurodegenerative diseases, cancer, stem cell-based therapies and many other diseases, and may stimulate the development of new pharmacological strategies to combat these diseases.

Keywords: Ca²⁺/cAMP; Signaling interaction/clinical research

knowledge from basic science to approaches for treating human diseases-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 Ca²⁺ ions in the cytosol ([Ca²⁺]_c) serve as a messenger signal to couple the stimulus to muscle contraction, or to neurotransmitter release, among other physiological responses [1,2]. A huge number of experiments performed since the discovery of the role of Ca²⁺ in the control of the heart beat [3] have set the dogma that in excitable cells, the increased Ca²⁺ influx by voltage-activated Ca²⁺ channels (VACC) elicited by depolarizing stimuli, triggers muscle contraction and the release of neurotransmitters, and hormones. Conversely, the mitigation of Ca²⁺ influx produced by VACC blockers causes a reduction of those responses [4,5].

The above concepts imply that the enhanced Ca²⁺ entry during cell depolarization and/or enhanced Ca²⁺ release from the sarco-endoplasmic reticulum (ER) augments the (Ca²⁺)_c and the triggering of the contractile, or secretory responses. However, about four decades ago, a study showed that verapamil at low concentrations produced a paradoxical increase of the contractions mediated by sympathetic nerves from vas deferens [6]. On the other hand, nifedipine was recently found to paradoxically augment the exocytosis of catecholamine triggered by double-pulse depolarizations from voltage-clamped bovine adrenal chromaffin cells, another interesting model to study sympathetic neurotransmission [7]. How these two L-type VACC blockers can enhance, instead of reducing, the Ca²⁺-dependent responses of contraction, and secretion? We properly gave a response to this “calcium paradox” in 2013 through the Ca²⁺/cAMP signaling interaction [8].

Role of Ca²⁺/cAMP signaling interaction in clinical research

In the vas deferens, both release and postsynaptic actions of noradrenaline (NA), and other neurotransmitters such as adenosine 5' triphosphate (ATP), depend on Ca²⁺ influx by VACC, and the ensuing elevations of (Ca²⁺)_c [9]. Hence, some authors found that verapamil abolished both noradrenergic and purinergic components of the sympathetically-mediated contractions of the vas deferens [10,11]. In an old report, however, it was showed that verapamil inhibited the

Introduction

Nowadays, an emerging concept in clinics is the “translational research”. Basically, “translating” current

sympathetically-mediated contractions of the rat smooth muscles (vas deferens), as predictable; nevertheless, this report also described that the low concentrations of verapamil produced a surprising increase of those contractions [6]. This paradoxical effect was corroborated in 1981 by French and Scott [12], also in the sympathetically-mediated contractions of the vas deferens. Furthermore, six years later a third study reported that verapamil and diltiazem increased the sympathetically-mediated contractions of the vas deferens; this result was attributed to an agonist effect of CCB on L-type VACC, thus increasing Ca^{2+} influx and neurotransmitter release [13]. Another published report (two years later) revealed that both, L- type VACC blockers and activator BAY K 8644, elicited similar increases of the sympathetically-mediated contractions of the smooth muscles (vas deferens); the authors did not provide a reasonable explanation for such paradoxical observation [14].

In a study from our laboratory, we could replicate those previous observations in the sympathetically-mediated contractions of the rat vas deferens: low verapamil concentrations produced a small increase in muscle contraction, while high CCB concentrations caused full inhibition of contractions [8]. Similar to the effects observed with high concentrations of CCB, various cAMP enhancers (e.g. the phosphodiesterase inhibitor rolipram) and adenylyl cyclase (AC) activators (e.g. forskolin) also reduced sympathetically mediated contractions; however, low verapamil concentrations in the presence of cAMP enhancers caused a significant increase of muscle contractions. The increased muscle contraction can in turn be reduced by adding an AC inhibitor, SQ22536, to lower the cytosolic cAMP level. These findings suggest that interactions in the Ca^{2+} /cAMP signaling pathways could play a key role in explaining the "calcium paradox" as observed in the presence of CCB and cAMP enhancers [8]. Thus, these findings can dramatically impact on the cardiovascular, neurodegenerative disorders, cancer and other diseases [15-17].

Based on these findings, we have anticipated that the pharmacological modulation of the Ca^{2+} /cAMP signaling interaction by combined use of the L-type CCB 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 neuroprotector strategy opens a novel pathway for the drug development more efficient and safer for the therapy of several neurodegenerative diseases, including Alzheimer's, Parkinson's, amyotrophic lateral sclerosis and Huntington's diseases [15,16]. **Figure 1** illustrates how the pharmacological modulation of the Ca^{2+} /cAMP signaling interaction could be used in human therapy.

The Ca^{2+} /cAMP signaling interaction can be pharmacologically modulated by combined use of drugs that reduce (Ca^{2+})c such as CCB, and cAMP-enhancer compounds such as PDE inhibitors and AC activators. This pharmacological modulation could be a new strategy to attenuate neuronal death caused by cytosolic Ca^{2+} overload and to increase neurotransmitter release. L, N, PQ: Ca^{2+} channel types; PDE:

phosphodiesterase; RyR: ryanodine receptors; IP3R: IP3 receptors; SERCA: sarcoendoplasmic reticulum Ca^{2+} -ATPase; (+): stimulation; dotted arrow: weak effect; solid arrow: strong effect.

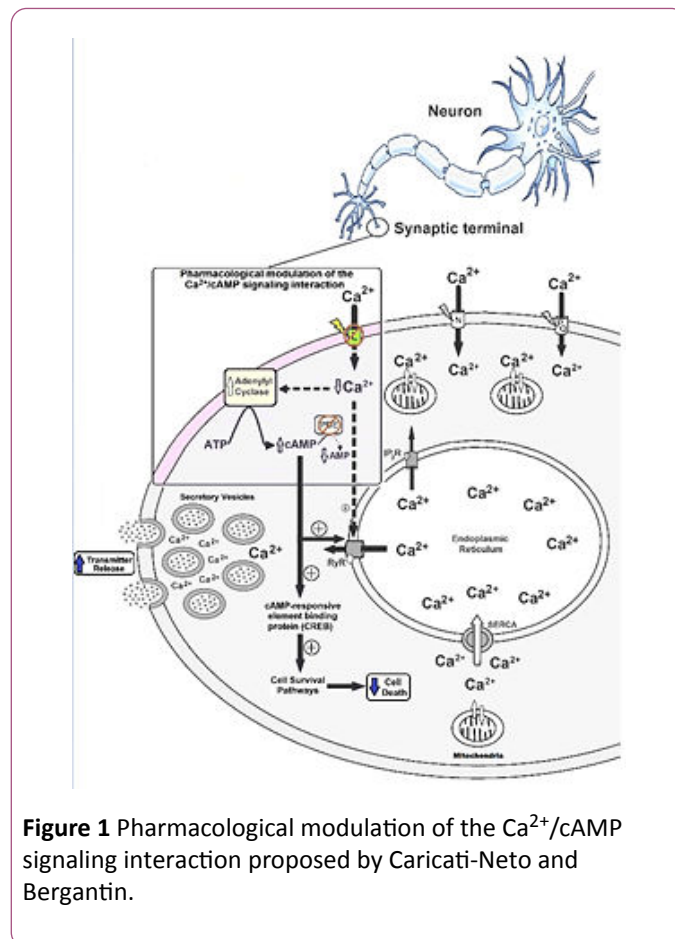


Figure 1 Pharmacological modulation of the Ca^{2+} /cAMP signaling interaction proposed by Caricati-Neto and Bergantin.

In fact, it was demonstrated that the prescription of L-type CCBs reduces motor symptoms, and reduces progressive neuronal death in animal model of Parkinson's disease, indicating that L-type CCBs are potentially viable neuroprotective pharmaceuticals [18]. 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 [19]. These results for the neuroprotective effects of CCBs have been reinvestigated in thousands elderly hypertensive patients with memory dysfunction [20]. These studies concluded that patients who have taken CCBs had their risk of cognitive dysfunction decreased, such as Alzheimer's disease [20]. These findings reinforce the idea that reduction of cytosolic Ca^{2+} overload produced by L-type CCBs due to blockade of Ca^{2+} influx could be an alternative pharmacological goal to reduce, or prevent, neuronal death in neurodegenerative diseases.

Due to involvement of the Ca^{2+} and cAMP signaling pathways in the regulation of the cellular differentiation process, it is possible that the pharmacological modulation of the Ca^{2+} /cAMP signaling interaction could stimulate the cellular differentiation in stem cells [15,16]. In this case, this

pharmacological strategy could be used in the cell therapy for the treatment of cardiovascular and neurodegenerative diseases.

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 drugs targets for cancer therapy [17]. With this concept in mind, some studies showed that drugs able to interfere with the intracellular Ca^{2+} signaling, such as L-type CCB, inhibit proliferative response in different cancer cells. In addition, 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 [17]. Then, the pharmacological modulation of the Ca^{2+} /cAMP signaling interaction in the cancer cells may represent a new therapeutic strategy for cancer progression.

Conclusion

The Ca^{2+} /cAMP signaling interaction may dramatically impact on medical research and therapeutics, stimulating the development of new pharmacological strategies for the therapy of human diseases, including: cancer, cardiovascular diseases, neurodegenerative diseases, and yet cellular therapy using stem cells.

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