The “Calcium Paradox” and its Impact on Neurological and Psychiatric Diseases (Second Edition)

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By

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Cambridge
Scholars
Publishing

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This book first published 2018

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN (10): 1-5275-1377-7
ISBN (13): 978-1-5275-1377-8

I dedicate this book to my parents: Maria Lúcia and Armando Magno; *amare et sapere vix deo conceditur*. I also dedicate this book to my aunt Maria de Fátima and to my brother Lucas, and to my PhD mentor Afonso Caricati-Neto from UNIFESP (Brazil).

Dedication by Leandro Bueno Bergantin, Ph.D.

I sincerely dedicate this book to my wife Mara, to my daughters Bruna and Bianca, and to my friend and colleague Leandro Bueno Bergantin from UNIFESP (Brazil).

Dedication by Afonso Caricati-Neto, Ph.D.

“In that rainy afternoon, the young researcher and his supervisor were preparing their daily experiment. On its course, there was a remaining solution containing Verapamil, a classical L-type CCB. In a relapse, the young researcher decided to add this solution (containing CCB) in an isolated smooth muscle. There was no apparent reason for it! The smooth muscle was prior relaxed with a drug (rolipram) that increased the cAMP cytosolic concentration. Putatively, addition of verapamil in the isolated smooth muscle should enhance (much more) the relaxation of the muscle contractions sympathetically-mediated! To his surprise, it was like that: the young researcher witnessed a drastic contraction of the smooth muscle! Puzzled with what he had observed, the young researcher and his supervisor did not know the impact and the magnitude of their discovery until that time”

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PREFACE

This book extends the original concept of the "calcium paradox" discovery, herein compiling more than 300 references (including > 30 of our own authorship) in the field of Ca^{2+}/cAMP signalling pathways, neurotransmission and neurodegeneration, and neurological and psychiatric diseases. In this second edition, novel illustrations and tables (highlighting current medicines for treating these diseases, including their mechanism of action) have improved the understanding of the concepts for students and clinicians. The description of other neurodegenerative diseases (Spinal muscular atrophy, Amyotrophic Lateral Sclerosis and Huntington´s disease) has added novel insights for treating these diseases. The growing increment in the life expectancy of the world's population has increased the concern about the age-related neurological diseases (neurodegenerative diseases), such as Alzheimer's (AD) and Parkinson's (PD) diseases, and others. It is now well recognized that an imbalance of intracellular Ca^{2+} homeostasis contributes to the pathogenesis of neurological diseases such as the neurodegenerative diseases, including AD and PD, and others. Healthy brain aging can be promoted by regular exercise, moderation in caloric intake and engaging in intellectually challenging activities. These lifestyle factors may stabilize the neuronal Ca^{2+} homeostasis. Our discovery of the role of "calcium paradox" due to the interaction between Ca^{2+} and cAMP signalling pathways (Ca^{2+}/cAMP interaction) in the neurotransmission, and neuroprotection, has subsidized the understanding of the pathophysiology, and pharmacology, of the neurological and psychiatric diseases, opening a large pathway for the advancement of new pharmacological strategies (more effective) for the treatment of these diseases. Our proposal involves pharmaceuticals already approved, and clinically safe, from non-neurodegenerative therapy indications (hypertension). Thus, combined with improvements in the lifestyle factors, it may allow sustained increments in the life quality of age-related neurological patients. Finally, this book briefly discusses other aspects of the Ca^{2+}/cAMP signalling pathways, such as their possible implication on cancer.

ACKNOWLEDGMENTS

Thanks to the continued financial support from CAPES, CNPq and FAPESP (Bergantin´s Postdoctoral Fellowship FAPESP #2014/10274-3). Also, thanks to Elsevier - “author use” - Reuse of portions or extracts from the article in other works - http://www.elsevier.com/journal-authors/ author-rights-and-responsibilities#author-use.

LIST OF MAIN ABBREVIATIONS

AC	adenylyl cyclases
ACh	acetylcholine
AD	Alzheimer´s disease
ALS	Amyotrophic Lateral Sclerosis
BAY K 8644	Ca^{2+} channels activator
BP	blood pressure
Ca^{2+}	calcium ion
CCB/CCBs	Ca^{2+} channel blockers
CICR	Ca^{2+} -induced Ca^{2+}-release
ER	endoplasmic reticulum
HD	Huntington´s disease
IBMX	3-isobutyl-1-methylxanthine
IP_3R	inositol trisphosphate receptor
IRBIT	IP_3R binding protein released with IP3
LDCV	large dense-core vesicle
MIT	mitochondria
NA	noradrenaline
NCX	Na^{+}/Ca^{2+} exchanger
PD	Parkinson´s disease
PDE	phosphodiesterases
PKA	protein kinase cAMP-dependent
PKC	protein kinase Ca^{2+}-dependent
RRP	ready-release-vesicle-pool
RyR	ryanodine receptors
SERCA	sarcoendoplasmic Ca^{2+}-ATPase
SHR	spontaneously hypertensive rats
SMA	Spinal muscular atrophy
SQ 22536	adenylyl cyclase inhibitor
SV	synaptic vesicles
VACC/VACCs	voltage-activated Ca^{2+} channels
VD	vascular dementia

CHAPTER ONE

INTRODUCTION

Analyzing Medline database from 1975 to 1996, Grossman and Messerli (1) found 63 clinical studies, involving 1,252 hypertensive patients, reporting alterations of the sympathetic activity produced by an acute and chronic administration of the L-type calcium (Ca^{2+}) channel blockers [CCB or pharmacologically known as voltage-activated Ca^{2+} channels (VACC) blockers such as verapamil, diltiazem and nifedipine: an important class of drugs largely used for the antihypertensive therapy by decreasing the arterial pressure due to the reduction of Ca^{2+} entry both into the cardiac and smooth muscle cells, and into the sympathetic neurons]. Grossman and Messerli´s study (1) showed that the acute administration of the CCB positively produced a significant reduction of the mean arterial pressure, but negatively produced a significant increment of plasma noradrenaline (NA) levels (sympathetic hyperactivity), and an increase of heart rate. This study showed that these adverse effects of the CCB (sympathetic hyperactivity) could be directly involved in the increase in morbidity, and mortality, associated to the chronic use of these drugs. However, the cellular and molecular mechanisms involved in this CCB-induced sympathetic hyperactivity remained unclear for several decades, or even more!

Using tissues richly innervated by sympathetic nerves as an *ex vivo* study model of the sympathetic neurotransmission, such as rodent vas deferens (2-7), several studies showed that verapamil produced paradoxical effects on the electrically-evoked neurogenic contractions mediated by the sympathetic nerves (8, 9). These studies showed that in concentrations above 1µM, verapamil inhibited these neurogenic contractions, but paradoxically potentiated these contractions in concentrations below 1µM (10-13).

In fact, since 1975 it was reported that, despite the well-known effect of verapamil to block the sympathetically mediated contractions of the smooth muscles (vas deferens), lower concentrations of verapamil caused a surprising augmentation of those contractions (10). In agreement with this, French & Scott (1981) (11) observed that verapamil unexpectedly

potentiated the neurogenic contractions in the prostatic portion of vas deferens, but antagonized those of the epididymal portion. These authors provided no reasonable explanation for this paradoxical finding. Furthermore, six years later (12), another study reported that verapamil and diltiazem enhanced the purinergic-mediated neurogenic twitch responses of the electrically-stimulated rat vas deferens; the authors attributed this effect to an unrealistic agonist effect of verapamil on the presynaptic L-type VACC, thus enhancing the Ca^{2+} entry and the ATP release (12). From these reports, we may already suggest that this paradoxical effect relies on increases in the secretory activity (response) of the sympathetic nerves. Two years later (1989), a fourth study appeared showing that both, L-type VACC blockers and activator BAY K 8644, elicited similar augmentations of the sympathetic contractions of the entire electrically-stimulated mouse vas deferens (13). Most interesting, these authors observed that verapamil (30 μM) markedly enhanced the potentiation caused by Bay K 8644 in a supra-additive fashion, suggesting that verapamil and Bay K 8644 can enhance the neurogenic contractions by different mechanisms, discrediting the hypothesis of an agonist effect of verapamil on the presynaptic L-type VACC.

In a recent study from our laboratory (see figure 1-1), we could reproduce those earlier observations in the neurogenically-induced contractions of the rat vas deferens: at lower concentrations, verapamil elicited a tiny augmentation, while at higher concentrations the VACC blocker caused a full inhibition of the contractions (6, 14, 15) . The interesting 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, depressed the neurogenic vas deferens contractions; however, in the presence of cAMP enhancers, the lower concentrations of verapamil caused a drastic augmentation of the neurogenic contractions mediated by the endogenously released ATP. The inhibition of AC by SQ 22536 attenuated the enhanced contractions, suggesting that an interaction of the Ca^{2+}/cAMP intracellular signalling pathways (Ca^{2+}/cAMP interaction) could, perhaps, explain the paradoxical effects of the combined drugs, verapamil and cAMP enhancers (6) .

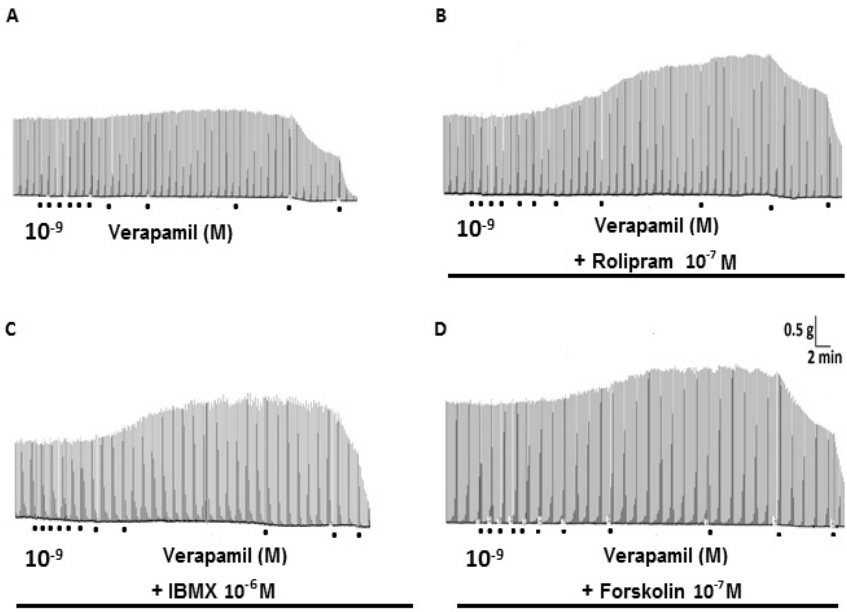

Figure 1-1 Effect of verapamil and agents that increased [cAMP]c (rolipram, IBMX and forskolin) on the neurogenic contractions mediated by the sympathetic nerves in rat vas deferens. Note in (A) that Verapamil, in high concentrations (>10^{-6} M), inhibited neurogenic contractions due to the reduction of neurotransmitter release, but discreetly potentiated these contractions in low concentrations (<10^{-6} M). Pre-incubation of tissue (15 min) with agents that increased [cAMP]c reversed verapamil inhibitory effect, resulting into potentiating neurogenic contractions (B, C and D). According to theoretical model proposed by Bergantin et al., 2013: the reduction of the calcium influx due to the L-type VACC blocker (verapamil), combined with an increase of cAMP, resulted in an increase of the neurotransmitter release from the sympathetic nerves of vas deferens. (Extracted from Bergantin et al., 2013 Cell Calcium – http://www.sciencedirect.com/science/article/pii/S0143416013000894).
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On the basis of the classical receptor theory, the combination of two drugs with an inhibitory action produces inhibitory effects (16). Thus, the potentiation of the neurogenic contractions of the rat vas deferens by a simultaneous administration of verapamil and [cAMP]c enhancers is an experimental finding unexpected in accordance with the receptor theory. The interaction between the intracellular signalling pathways mediated by cAMP and Ca^{2+} could explain in a more consistent way this pharmacological phenomenon. The idea of this interaction was supported by various experimental protocols.

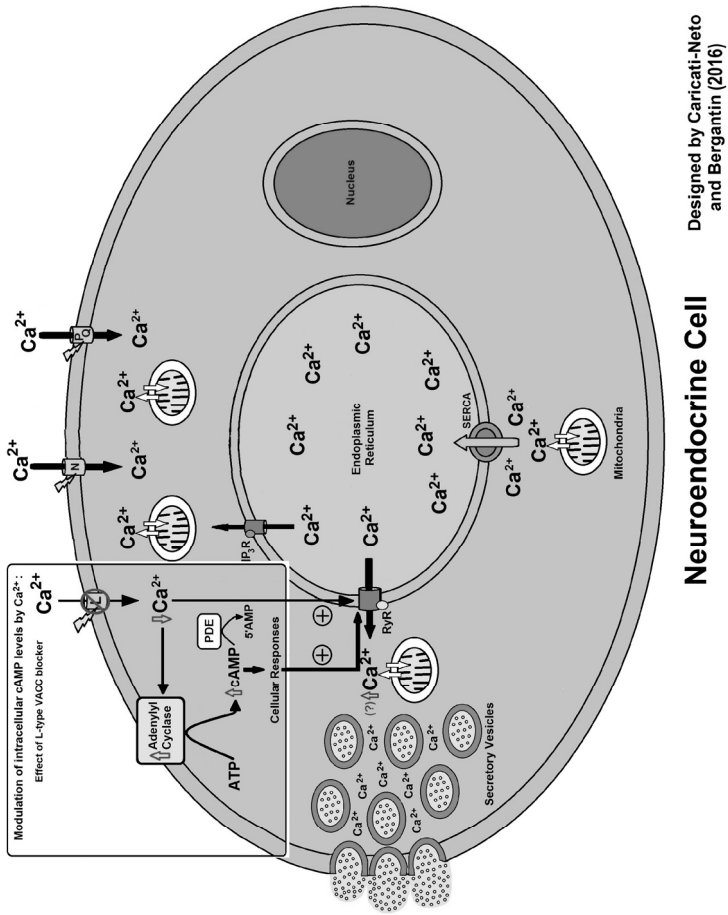
Bergantin et al. (6) showed that the potentiation of the smooth muscles neurogenic contractions produced by the combination of verapamil, and enhancers of cAMP cytosolic concentration ([cAMP]c), was prevented by a reduction of [cAMP]c caused by the AC inhibitor SQ 22536, or by depletion of the Ca^{2+} storages from the endoplasmic reticulum (ER) by the sarcoplasmic ER Ca^{2+} reuptake blocker thapsigargin (6). These findings suggest that a blockade of the Ca^{2+} influx through the L-type CCB, by verapamil, produces a reduction of $[Ca^{2+}]c$, leading into the increase of AC activity, that in turn results in an increase of [cAMP]c. The increase of [cAMP]c stimulates the Ca^{2+} release from the ER, and consequently increments cellular responses, as shown in figure 1-2.

Considering that drugs which increase the cAMP levels (rolipram, IBMX and forskolin) classically have relaxant effects in the smooth muscles, mainly through the inhibition of phosphorylation of the smooth muscle myosin (17), and that high concentrations of the L-type VACC blockers inhibit the sympathetic neurotransmission, the result we obtained was clearly unexpected: the combination of these drugs produced a drastic potentiation of the neurogenic contractions, instead of the expected inhibition (figures 1-1 and 1-2). Obviously, these results cannot be attributed to an artifact, considering that by using multiple combinations of the drugs (e.g. rolipram plus verapamil, IBMX plus verapamil, etc.), the paradoxical phenomenon still existed. Based on this intriguing result, we built up the "calcium paradox" theory, trying to explain the enigma that existed in the sympathetic transmission since 1975 (figure 1-2).

As discussed above, it is amply documented that an increase of [cAMP]c causes the relaxation of the smooth muscle (17); this is also true for the L-type VACC blockers. Thus, by using the compounds that augment [cAMP]c and VACC blockers separately, their predominant effect could be exerted directly into the smooth muscle (postsynaptic), causing its relaxation. However, at the presynaptic level (secretory apparatus, figure 1-2), low concentrations of the VACC blockers, as well as agents that produce an increase of [cAMP]c, may have excitatory

effects on the neurotransmission, and other cellular responses (18). The combination of these drugs caused a synergistic effect through the Ca^{2+}/cAMP interaction at this level, then predominating the presynaptic effect, and thus enhancing the transmitter release, resulting in an increase of muscle contraction (figures 1-1 and 1-2).

A



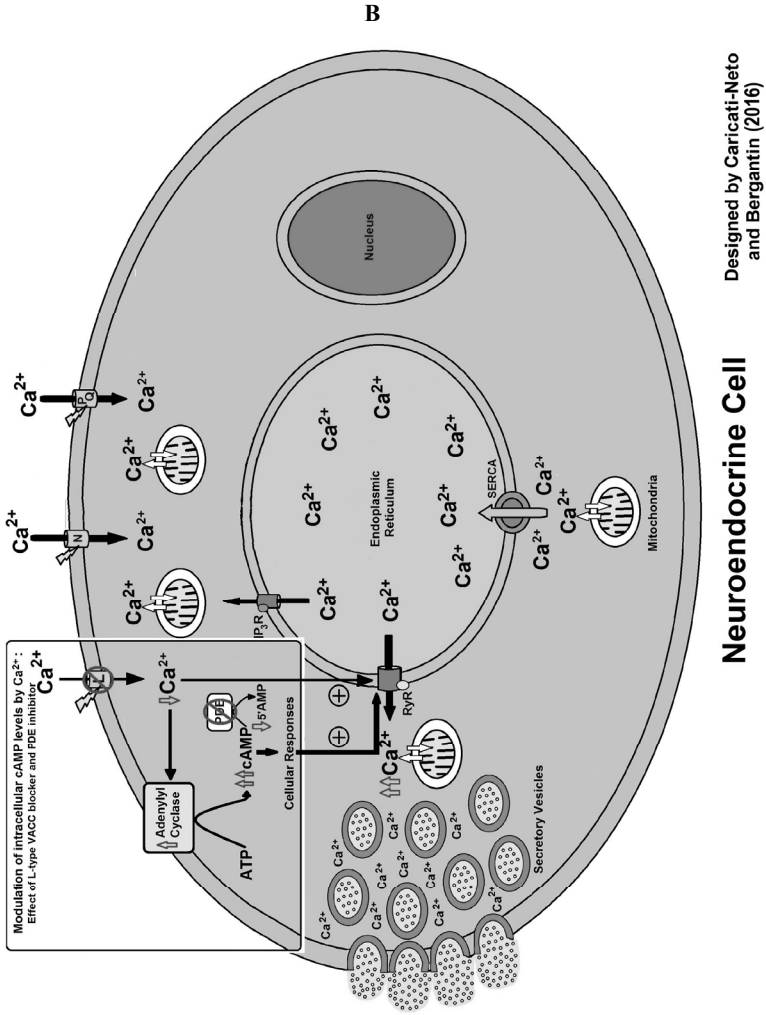


Figure 1-2 By reducing the Ca^{2+} influx, and consequently the cytosolic Ca^{2+} concentration $[Ca^{2+}]_c$, the L-type CCB should reduce a secretory response. However, the reduction of Ca^{2+} entry through the L-type CCB, verapamil or nifedipine, may activate the Ca^{2+} – sensitive adenylyl cyclase (AC), thereby causing the activation of the cAMP pathway – Ca^{2+} release from the endoplasmic reticulum (ER) (A). Thus, in this model we have two "antagonistic forces" driven by Ca^{2+} entry and cAMP: the channel component (fast activity), and the component of the signalling pathway (slow activity). The "calcium paradox" implies a secretory cell reduction of Ca^{2+} entry produced by the low verapamil

concentrations, removal of Ca^{2+} dependent inhibition of AC and/or phosphodiesterase (PDE) colocalized with L-type CCB, augmented intracellular levels of cAMP, an increased ER Ca^{2+} release via RyR, and an enhanced release of secretory vesicle (B).

It seems now clear that the "calcium paradox" occurs when using low concentrations of the VACC blockers (10-13,19). We try to explain this fact in figure 1-2, where two components associated to the L-type VACC blockers are shown: 1) the component of a channel (fast activity) and 2) the component of a signalling pathway (slow activity). At low blocker concentrations, it is plausible that the component of the signalling pathways is strong enough to overcome the effect of a mild VACC inhibition. Also, in results from our lab performed in bovine adrenal chromaffin cells (secretory response activity), we could clearly see this phenomenon: nifedipine in low concentrations may enhance their secretory activity (20). In addition, it is plausible that the biphasic effect of BAY K 8644 on the neurogenic contraction (concentration-dependent contraction and relaxation) (21) and secretion (22) could also be explained in the context of the "calcium paradox". At higher concentrations, the intensive Ca^{2+} influx promoted by BAY K 8644 may inhibit the constitutive activity of the Ca^{2+} and cAMP signalling pathways associated to the L-type VACC, thus reducing the secretory response mediated by a Ca^{2+} release from the ER (figure 1-2).

The concept of the complex cAMP-IP_3R interaction as a "third messenger", which may mediate the synergistic action of the Ca^{2+} and cAMP signalling, is now emerging (23). Recent data suggest that IRBIT (Inositol-trisphosphate (IP_3) receptors binding protein released with IP3) may become a central-stage in the mechanism mediating the synergism between cAMP and Ca^{2+} signalling pathways, by functioning as a "third messenger", which favors the crosstalk between IP_3R and other proteins. Another central component is the classical phosphorylation by the protein kinase cAMP-dependent (PKA) of IP_3R. Thus, IP_3Rs, IRBIT, PKA and the effector proteins have to be assembled into microdomains to allow the efficiency of IRBIT. In resting cells, when cellular IP_3 levels are low, IRBIT is bound to the IP_3R; thus IP_3R functions to buffer the availability of free IRBIT (23). Increases in cAMP levels may lead into the dissociation of IRBIT from IP_3 receptors, and its translocation into effector proteins located either at intracellular organelles and/or the plasma membrane; in this manner, IRBIT functions as a "third messenger" that transmits the information carried out by the second messengers cAMP and IP_3. At the same time, IRBIT integrates and synergizes the activity of the cAMP and Ca^{2+} signalling systems, providing a molecular mechanism for

the synergistic action between them. We think this "idea" fits into the "calcium paradox" hypothesis; in fact, Ca^{2+} release from the ER into the cytosol, triggered by verapamil plus rolipram in rat chromaffin cell slices, was blocked upon an ER Ca^{2+} depletion with thapsigargin (6). Furthermore, considering that this "calcium paradox" could also explain data from different biological systems (24,25), it is becoming apparent that the enigma of the "calcium paradox" in the context of neurotransmission, and neurosecretion, may be resolved through the Ca^{2+}/cAMP interaction. However, further work is needed to clarify this challenging hypothesis.

As in vas deferens, some paradoxical effects have also been recently reported to occur in adrenal chromaffin cells, another interesting model of neuroendocrine cell. For instance, in a study performed in voltage-clamped bovine chromaffin cells, the blockade of the L-type VACC with nifedipine transformed the exocytotic responses, elicited by a double-pulse protocol, from a depression into a facilitation (20). In an earlier study, it was shown that nifedipine suppressed the endocytotic response triggered by a long depolarizing stimulus (26). The explanation for the paradoxical effect of nifedipine could rest in the fact that the inhibition of a rapid endocytosis triggered by Ca^{2+} entry, through the L-type VACC of bovine chromaffin cells (α_{1D}, Cav 1.3), could unmask a full exocytotic response. A second explanation may lay in the observation that a Ca^{2+} entry through L-type VACC causes the inhibition of P/Q type VACC (α_{1A}, Cav 2.1) (27), that in chromaffin cells greatly contribute to the control of the secretory response (28). By blocking the L-type VACC, nifedipine could remove the Ca^{2+} dependent inactivation of P/Q-type VACC to enhance a Ca^{2+} entry through them, and thereby augmenting the secretory response.

An additional explanation for the nifedipine paradoxical effect in chromaffin cells (20) could be found in the context of the "calcium paradox" described in the vas deferens, and in the Ca^{2+}/cAMP interaction (6). In agreement with these observations, recent reports (29,30) have observed an inhibitory effect of an extracellular Ca^{2+} on Ca^{2+} - dependent exocytosis. These apparent paradoxical findings may be explained in the context of the "calcium paradox" described in the vas deferens, and in the Ca^{2+}/cAMP interaction (6) (figure 1-3).

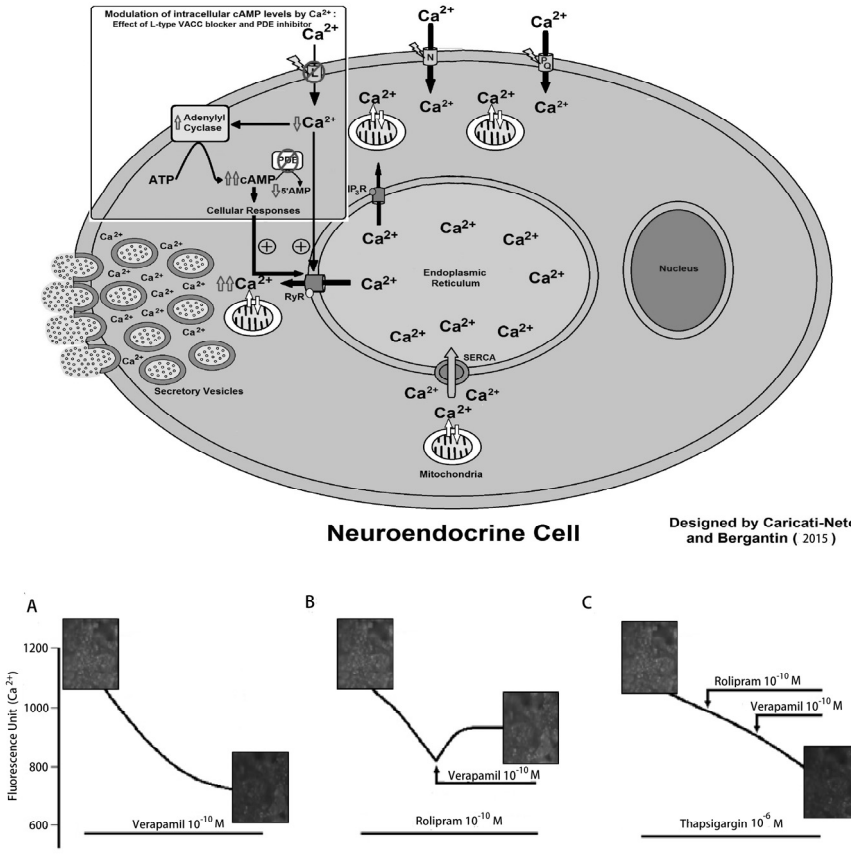


Figure 1-3 Effect of verapamil and rolipram on fluorescence associated to Ca^{2+} indicator (fura-2) in adrenal medullar slices. Verapamil reduced fluorescence unit by a reduction of Ca^{2+} influx due to the L-type VACC blocker (A). However, the pre-incubation of slices with rolipram produced a reversed effect of the verapamil effect, resulting into an increase of fura-2 fluorescence (B). Thapsigargin abolished this effect, indicating that the endoplasmic reticulum participates in this mechanism (C). According to the theoretical model proposed by Bergantin et al., 2013: the reduction of calcium influx due to the L-type VACC blocker (verapamil) combined with an increase of cAMP, due to a PDE inhibition (rolipram), resulted in an increase of catecholamine release from adrenal chromaffin cells due to an increase of Ca^{2+} cytosolic transients. (Adapted from Bergantin et al., 2013 Cell Calcium –

Thus, in the light of the paradoxical effects of the combined drugs, verapamil plus rolipram in the vas deferens, it could be possible to implicate cAMP also in the paradoxical effects of nifedipine in the secretory response of chromaffin cells. In fact, several reports have been published on the role of cAMP in the regulation of neurotransmitter release, as well as in the postsynaptic actions of different neurotransmitters (7). Additionally, the release of sympathetic transmitters is regulated both by Ca^{2+} and cAMP (31-35). For example, in 1987 Morita and colleagues (36) observed that forskolin enhanced both norepinephrine and endogenous catecholamine release evoked by 30 mM K^+ from chromaffin cells. The effects of forskolin were substantial when catecholamine release was evoked with low concentrations of acetylcholine (ACh), or excess of K^+. Forskolin also enhanced the catecholamine release induced by ionomycin and veratrine, or by caffeine in Ca^{2+}-free medium. The potentiation by forskolin of the ACh-evoked catecholamine release was manifested in low Ca^{2+} concentrations in the medium but decreased when Ca^{2+} concentration was increased. These results clearly do not respect the concept that the mitigation of a Ca^{2+} entry produced by CCB and/or decreased of Ca^{2+} medium concentration causes a diminution of cellular responses (37,38). Finally, these authors (39) suggested that cAMP could increase the stimulation-induced catecholamine release by enhancing the Ca^{2+} uptake across the plasma membrane and/or altering the Ca^{2+} flux in an intracellular Ca^{2+} store.

In this point of view, some studies have also shown a positive correlation between an elevation of $[cAMP]_c$ and the catecholamine release in bovine chromaffin cells, stimulated with nicotine (40), PACAP (41), histamine (42) or VIP (43); this is also true for the PDE inhibitors rolipram or IBMX (44-46).

To enhance the secretion, cAMP may act at several targets, including the VACC of chromaffin cells, the regulation of the size of subplasmalemmal vesicle pools and/or the kinetics of the fusion pore during the last steps of exocytosis (44). It is well established that the L-type VACC is the most sensitive to cAMP and PKA (47-49). For example, in mouse chromaffin cells, rolipram augments both $[cAMP]_c$, L-currents and the secretion (18). Also, rolipram increased the size of the ready-release-vesicle-pool (RRP) (50) by 75%, nearly doubled the membrane area of single vesicles in rat chromaffin cells (48), and augmented the

quantal size by 38% also in rat chromaffin cells (51). Furthermore, the AC activator forskolin enhanced by 50%, and rolipram by 25% the quantal size of single vesicles in bovine chromaffin cells (44). On the other hand, in mouse chromaffin cells, rolipram increased more the size of the RRP (47%) than the quantity of Ca^{2+} penetrating into the cell (16%); this suggests that about 30% of the increased secretion is Ca^{2+}-independent and occurs down-stream of a $[Ca^{2+}]_c$ elevation through the L-type VACC, most likely by affecting directly the secretory apparatus (18). However, as in the vas deferens, Ca^{2+}/cAMP interaction may also occur in chromaffin cells; an evidence for such interaction is still actually lacking. Whether the paradoxical effects of nifedipine could be explained in the context of the "calcium paradox", emanated from the vas deferens, deserves more experimental attention. Dysregulation of this Ca^{2+}/cAMP interaction could lead into serious pathological consequences, such as cardiovascular dysfunctions.

Stressful situations like fear, cold, severe hypoglycemia, hemorrhage, and acute myocardial infarction trigger the release of neurotransmitters from postganglionic sympathetic neurons (ATP and noradrenaline) and hormones from chromaffin cells of adrenal medulla (ATP and adrenaline), which activate specific receptors located on the surface of effectors cells (smooth and cardiac muscles, and exocrine cells), stimulating the coordinated physiological response that prepares mammals´ body to survive by combating an enemy, or to flee from danger. Firstly described as "*fight or flight*" responses by American physiologist Walter Bradford Cannon at the beginning of the twentieth century (52), these physiological reactions to stress mediated by the sympathetic nervous system, and controlled by the central nervous system nuclei at the cortex, hypothalamus and spinal cord are vital to the survival of mammals, including humans. Thus, the heart rate, the strength of myocardial contraction and blood pressure increase; the blood flow switches into the skeletal muscle; glucose is mobilized from the liver and rises into the circulation; and the pupils and bronchioles dilate (52).

Several experimental and clinical studies, performed since 1950 decade, have shown that alterations in the activity of sympathoadrenal axis, and the subsequent rate of catecholamine release, are involved in the pathogenesis of the cardiovascular diseases, including systemic arterial hypertension in humans and in animal models, such as *Spontaneously Hypertensive Rats* (SHR) (53-57). For example, a sympathetic hyperactivity characterized by a significant elevation of circulating levels of noradrenaline, and adrenaline, due to catecholamines hypersecretion by medullary adrenal tumor is involved in the severe hypertensive crisis in

humans with pheochromocytoma (malign tumor of adrenal medulla) (57). A similar sympathetic hyperactivity due to catecholamines hypersecretion by sympathetic neurons, and adrenal chromaffin cells, is involved in the pathogenesis of primary hypertension in humans and SHR (54-56). Although a catecholamine hypersecretion from sympathetic neurons, and adrenal chromaffin cells, constitutes a primary dysfunction responsible by arterial hypertension; and that the use of drugs that interfere with sympathoadrenal axis, such as blockers of α- and β-adrenoceptors, constitutes a classical strategy to treat human hypertension; the cellular and molecular mechanisms involved in this sympathetic dysfunction are yet unclear.

Due to the common embryologic origin with postganglionic sympathetic neurons in the neural crest (58), the adrenal chromaffin cells are of interest not only as the basis of the "*fight or flight*" response and sympathetic dysfunctions, but also because they have been excellent models to study the working of other secretory cells. Because of their unlimited availability, particularly from bovine species, and their ease of isolation and preparation in primary cultures (59), chromaffin cells have been widely used in biochemical, electrophysiological, and neuropharmacological studies. Their usefulness has been further enhanced by the development of techniques to separate noradrenaline- from adrenaline-containing vesicles (60, 61). Thus, fundamental findings on the catecholamine synthesis, storage, and release were extrapolated, with success, from these cells into basic neurotransmission mechanisms in the central and peripheral nervous systems.

Although the "*fight or flight*" response is complex, this response basically depends on a release of neurotransmitters, and hormones, synthesized and stored by postganglionic sympathetic neurons and adrenal chromaffin cells (52). The physiological function of the chromaffin cells consists in the exocytotic release of the catecholamines (noradrenaline and adrenaline) into the circulation in response to stressor stimuli (62). But, the demonstration that this secretory response was suppressed in the absence of extracellular Ca^{2+} became a clear notion that the release of hormone from neuroendocrine cells, and neurotransmitter from neurons, is tightly regulated with exocytotic fusion of secretory vesicles being triggered by a rise in $[Ca^{2+}]_c$ (50,63).

Because the exocytosis is a Ca^{2+}-dependent process (63), it is not surprising that chromaffin cells have been widely used as models to study the correlation between Ca^{2+} and exocytosis (50). They contain all the elements required for a strict control, both spatial and kinetic, of the Ca^{2+} transients required during the various steps of exocytosis in neuronal and