Anabolic Androgenic Steroids and Intracellular Calcium Signaling: A Mini Review on Mechanisms and Physiological Implications

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Abstract: Increasing evidence suggests that nongenomic effects of testosterone and anabolic androgenic steroids (AAS) operate concertedly with genomic effects. Classically, these responses have been viewed as separate and independent processes, primarily because nongenomic responses are faster and appear to be mediated by membrane androgen receptors, whereas long-term genomic effects are mediated through cytosolic androgen receptors regulating transcriptional activity. Numerous studies have demonstrated increases in intracellular Ca^{2+} in response to AAS. These Ca^{2+} mediated responses have been seen in a diversity of cell types, including osteoblasts, platelets, skeletal muscle cells, cardiac myocytes and neurons. The versatility of Ca^{2+} as a second messenger provides these responses with a vast number of pathophysiological implications. In cardiac cells, testosterone elicits voltage-dependent Ca^{2+} oscillations and IP_3R-mediated Ca^{2+} release from internal stores, leading to activation of MAPK and mTOR signaling that promotes cardiac hypertrophy. In neurons, depending upon concentration, testosterone can provoke either physiological Ca^{2+} oscillations, essential for synaptic plasticity, or sustained, pathological Ca^{2+} transients that lead to neuronal apoptosis. We propose therefore, that Ca^{2+} acts as an important point of crosstalk between nongenomic and genomic AAS signaling, representing a central regulator that bridges these previously thought to be divergent responses.

Keywords: Androgens, Ca^{2+}, cardiac myocytes, heart, genomic, neurons, nongenomic, skeletal muscle cells, testosterone.

1. INTRODUCTION

Second messengers are ions or molecules responsible for the transduction of extracellular signals into cellular processes. Among these, the Ca^{2+} ion is the most ubiquitous second messenger signal in eukaryotic systems and represents one of the most complex messages decoded by cells. Over the past decades, the study of intracellular Ca^{2+} has evolved from the observational study of Ca^{2+} transients inside different types of cells to becoming the basis of a vast array of physiological and pathological processes. For instance, we currently know that cardiac function depends almost entirely on fine-tuning of intracellular Ca^{2+}, which when imbalanced leads to arrhythmias, cardiac failure or stroke. In neurons, Ca^{2+} regulates many processes including gene expression or the synaptic release of vesicles. In these, and many other types of cells, the regulation of intracellular Ca^{2+} is key for the determination of life-death outcomes, which contribute to neuropathies, cell death or tumorigenesis. Therefore, understanding the physiology of Ca^{2+}-regulated processes is essential for finding new approaches to the treatment of human diseases including those of highest mortality in developed countries: cardiovascular disease, cancer and neurodegeneration, as well as many other genetic or endocrine disorders.

Steroidal hormones such as testosterone and its derivatives are well known for their androgenic properties and anabolic effects. The cumulative effect of these hormones is to direct the differentiation of organs and tissues toward the adoption of male phenotypes. Classically, anabolic androgenic steroids (AAS) act through binding to androgen receptors (AR), which once bound by their ligands, function as nuclear transcription factors promoting the expression of genes under the control of steroid-response elements (SRE). This programmed gene expression is achieved within a time course of hours after AAS binding to ARs. More recently, however, it has been described that steroidal hormones including AAS can also provoke faster responses, which do not involve gene expression. These effects have been termed as 'nongenomic', and they cover a wide range of intracellular processes such as the activation of

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1389-5575/11 $58.00+.00

membrane bound receptors, triggering of downstream pathways that involve protein kinases and phosphatases, mobilization of intracellular Ca^{2+}, as well SRE-independent changes in transcription [1]. The origin of these responses has been attributed to AR-AAS complexes present in caveolin-enriched zones of the plasma membrane [2], however, recent studies identify orphan candidates for membrane-bound AR that after binding to AAS, trigger activation of intracellular second messengers [3]. This fascinating emerging topic will certainly contribute to our understanding of the pathophysiological consequences of androgen action. In the next sections, we will focus our attention in those nongenomic actions of AAS that are related to Ca^{2+} signaling, with emphasis on such effects on cardiac muscle cells, skeletal muscle cells and neurons. We will discuss the mechanistic pathways involved, their pathological implications and their perspectives.

2. AAS AND CALCIUM

2.1. Brief Description of Cellular Ca^{2+} Toolkits

Cellular Ca^{2+} signals possess the unique features of spatial compartmentalization and temporality, which together determines the message they carry and therefore the response they evoke. For instance, very fast (milliseconds) and localized Ca^{2+} increases allow the fusion of presynaptic vesicles with the plasma membrane, leading to neurotransmitter release or receptor externalization. During muscle contraction, a series of complex, compartmentalized and sequential Ca^{2+} events provide a mechanism for the decoding of a depolarizing signal into the contraction of sarcomere fibers within fractions of seconds, a process known as excitation-contraction (EC) coupling. On another hand, Ca^{2+} waves propagated across the cytoplasm of many different cell types lead to the activation of Ca^{2+}-sensitive effectors, which include Ca^{2+}-binding proteins, Ca^{2+}-sensitive kinases and Ca^{2+}-sensitive transcription factors. Once initiated, these processes can operate in a temporal range that covers milliseconds, minutes and even hours in the case of gene expression. Such localized Ca^{2+} signals are tightly controlled by a highly specialized molecular toolkit, capable of increasing Ca^{2+} concentrations in specific zones of the cytosol or the nucleus, and of removing it very rapidly (for details please see [4]).

Briefly, Ca^{2+} can be either introduced to the cytosol from the extracellular medium, or released from internal Ca^{2+} stores such as the sarco-endoplasmic reticulum. Several different Ca^{2+} channels reside in the plasma membrane, and in response to a variety of stimuli, they increase their open probability state, allowing the influx of Ca^{2+} ions. Among these channels, the family of voltage dependent L-type Ca^{2+} channels is of interest here, because they are expressed in neurons, myotubes and cardiomyocytes, and constitute the first element to respond to a depolarizing signal. Upon opening, they produce localized cytoplasmic Ca^{2+} *sparklets*, which can be sensed by another Ca^{2+} channel resident in the endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR), known as the ryanodine receptor (RyR). In the case of neurons, type 1 RyRs respond by releasing Ca^{2+} from the ER in the form of *syntilla*, which allows the fusion of presynaptic vesicles with the plasma membrane and the release of neurotransmitters or hormones. In the case of cardiomyocytes, the opening of type 2 RyRs after a *sparklet* leads to SR Ca^{2+} release in the form of *sparks*, which stimulate the contraction of sarcomeres resulting in muscle contraction. In skeletal muscle and cultured myotubes, there is a direct interaction between L-type Cav1.1 channels and type 1 RyR facilitating the triggering of Ca^{2+} release.

Another ER-resident Ca^{2+} channel is the inositol 1,4,5-trisphosphate receptor (IP_3R). Unlike RyRs, these channels do not crosstalk with L-type Ca^{2+} channels. Instead, they are ligand-activated in response to inositol 1,4,5-trisphosphate (IP_3), which is produced in the inner face of the plasma membrane by the enzyme phospholipase C (PLC). Several isoforms of PLC exist, according to the type of plasma membrane receptor to which they are coupled. Once IP_3 is bound to IP_3Rs, these channels respond by releasing Ca^{2+} from the ER in the form of *puffs* into the cytosol and/or into the nucleus. These localized events lead to the activation of several effectors, including Ca^{2+}-binding proteins such as calmodulin, enzymes such as calcineurin and calmodulin kinase, as well as Ca^{2+}-sensitive transcription factors such as NFAT or CREB [5, 6]. Ca^{2+} can also be propagated throughout the cytoplasm in the form of *waves* through a crosstalk between aligned IP_3Rs, in which Ca^{2+} itself increases the opening probability of surrounding IP_3Rs.

The mechanisms to extrude Ca^{2+} from the cytosol include ATP-dependent pumps, such as the plasma membrane and the sarco-endoplasmic reticulum Ca^{2+} ATPases (PMCAs and SERCAs respectively), as well as the plasma membrane Na^+/Ca^{2+} exchanger (NCX). Mitochondria represent an additional Ca^{2+} buffering organelle, as a substantial load of Ca^{2+} released from the ER is driven into the mitochondrial matrix by the voltage-dependent anion channel (VDAC) and the mitochondrial Ca^{2+} uniporter (MCU). In this way, Ca^{2+} is removed rapidly from the cytosol, thus shaping an undetermined set of Ca^{2+} signals that encode a vast array of messages (Fig. **1**). For instance, slow Ca^{2+} signals in the cytoplasm and nuclei after electrical stimulation of skeletal myotubes depend on IP_3 receptors [7-9]. This slow Ca^{2+} signal is also modulated by mitochondria [10], and activates a specific program of gene expression. Equivalent signals have been observed in adult skeletal muscle fibers [11].

2.2. Acute Effects of AAS on Intracellular Ca^{2+}

Before focusing on the relationship between steroids and intracellular Ca^{2+}, one particular aspect of this relationship is the direct effect of AAS on Ca^{2+} channels. Increasing evidence indicates that L-type calcium channels are regulated by AAS in two different ways: acutely, by a direct antagonistic effect of AAS upon L-type Ca^{2+} channels, and chronically, after a genomic response that leads to increased expression of L-type Ca^{2+} channels [12, 13]. Relative to the structure-activity relationship, the Peers' group seems to have done the most research on this subject, using HEK 293Ar75 cells [14-16]. In a series of patch clamp experiments, among the different steroids tested, only testosterone blocked L-type calcium channels. There are three interesting observations to highlight these findings, the first one being that despite the structural similarity among all steroids assayed, only testosterone blocked the channel;

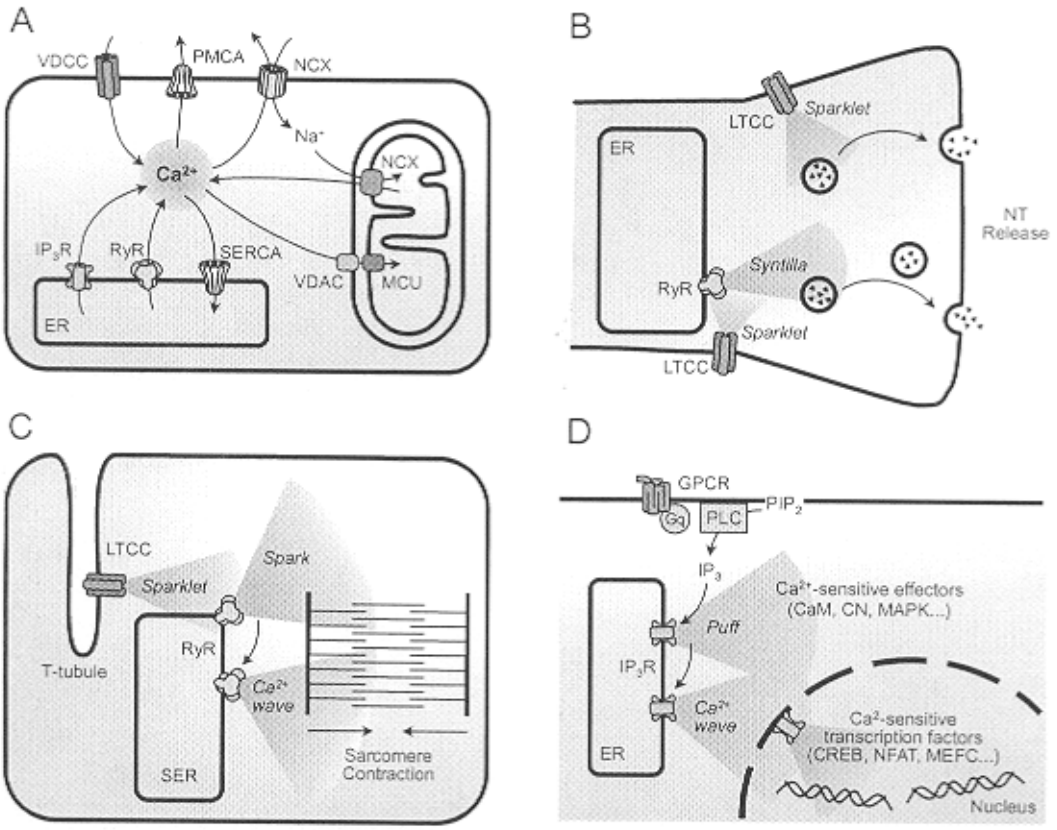

Fig. (1). Intracellular Ca^{2+} signaling at a glance. (**A**) General vision of the basic Ca^{2+} toolkit. Mechanisms that drive Ca^{2+} into the cytosol from the extracellular medium include ligand-gated Ca^{2+} channels (not shown) and voltage-dependent Ca^{2+} channels (VDCC). Ca^{2+}-release mechanisms from intracellular stores include inositol 1,4,5-trisphosphate receptors (IP_3R), ryanodine receptors (RyR) and the mitochondrial Na^+/Ca^{2+} exchanger (NCX). Mechanisms to extrude Ca^{2+} from the cytosol include the plasma membrane NCX, the plasma membrane and the sarco-endoplasmic reticulum Ca^{2+}-ATPases (PMCA and SERCA, respectively), as well as the mitochondrial outer membrane voltage dependent anion channel (VDAC) and the mitochondrial inner membrane Ca^{2+} uniporter (MCU). (**B**) Ca^{2+} microdomains regulate neurotransmitter release in neurons. L-Type Ca^{2+} channels (LTCC) introduce Ca^{2+} into the cytosol in the form of *sparklets*, which directly induce local vesicle fusion with the terminal, or activate RyRs to open and release higher amounts of Ca^{2+} from the endoplasmic reticulum (ER) in the form of *syntilla*, which elicits massive neurotransmitter release. (**C**) Ca^{2+} microdomains regulate sarcomere contraction in skeletal and cardiac muscle cells. Depolarizing signals in T tubules induce opening of LTCCs. The resulting *sparklet* stimulates the opening of RyRs in these cells, which release Ca^{2+} in the form of *sparks*. This Ca^{2+} increase propagates as Ca^{2+} *waves* to generate muscle contraction. (**D**) The general mechanism for IP_3R-mediated Ca^{2+} release in response to G protein-coupled protein receptors (GPCR) involves activation of phospholipase C (PLC) and local production of IP_3, which activates IP_3Rs to release high amounts of Ca^{2+} from the ER in the form of *puffs*. These signals lead to activation of Ca^{2+}-dependent effectors in the cytosol, including calmodulin (CaM), calcineurin (CN) and mitogen-activated protein kinases (MAPK), among others. The propagation of *puffs* also leads to Ca^{2+} *waves*, which encode different messages according to their amplitude and frequency. Nuclear IP_3R-mediated Ca^{2+} *puffs* or *waves*, lead to the activation of Ca^{2+}-dependent transcription factors, as illustrated.

second, considering the structural requirements, none of these structures resembles those of established Ca^{2+} channel blockers such as dihydropyridines, phenylalkylamines or benzothiazepines; and third, the researchers suggest that testosterone and nifedipine share common molecular requirements for inhibition of L-type Ca^{2+} channels in the S5 region of domain III [15]. It is important to note that the binding sites described for dihydropyridines are in segments 5 and 6 of domain III, and 6 of domain IV [17]. However, no specific binding region has been identified for AASs so far. Irrespective of these details, the acute effects of androgens upon L-type Ca^{2+} channels provide a potentially useful clinical strategy for the treatment of myocardial ischemia [18], coronary artery disease [19, 20], as well as for the reduction of cholesterol and visceral adiposity [21-23]. These effects resemble those of third generation dihydropyridines such as amlodipine; further structural studies will contribute to the elucidation of the direct mechanisms involved in these responses. Thus far, the effects of androgens on the cardiovascular system remain incompletely understood, and some studies point to increased cardiovascular risk associated with high circulating androgen levels [24, 25], which is related to genomic responses that lead to cardiomyocyte hypertrophy [26], high

L-type Ca^{2+} channel expression [12] and blood pressure [27, 28]. That said, other studies support a long-term cardioprotective role [29, 30]. Despite these differences, increasing clinical evidence supports consideration of transient use of testosterone derivatives to improve cardiovascular and pulmonary status [31].

2.3. Calcium-Mediated Myocyte Hypertrophy

The role of Ca^{2+} in mediating hypertrophy of cardiomyocytes and skeletal muscle cells has been well documented [32, 33]. Intracellular Ca^{2+} elevations trigger myocyte hypertrophic signaling *via* the phosphatase calcineurin, activation of the transcription factor NFAT [34] and the cyclic T-cdk9 complex [35]. Other transcription factors such as CAMTA2 are essential for regulating cardiac hypertrophy in response to calmodulin signaling, *via* the association with class II histone deacetylases (HDACs) [36]. Among the other Ca^{2+}-dependent transcription factors involved in myocyte hypertrophy is CREB [37] and MEF2C [38, 39]. Interestingly, the AR has also been shown to induce myocyte hypertrophy [40] and independently, to regulate the activity of MEF2C [41], which is also regulated by calcineurin [42]. Therefore, a reasonable and predicted link between these processes could be mediated by androgen-induced Ca^{2+} signaling. Interestingly, Ca^{2+} triggers cardiac hypertrophy in response to several other agents including angiotensin II, endothelin-1, α-adrenergic agents and mechanical stretching [43]. However, it is currently unknown how androgens regulate signal transduction cascades during cardiac hypertrophy, and Ca^{2+} is a prominent mechanism that could drive these effects in the heart and in skeletal muscle cells.

Nongenomic testosterone effects have been observed in skeletal muscle cells, *via* the release of Ca^{2+} from internal stores [44, 45]. This Ca^{2+}-*wave* signal involved a G protein-coupled membrane receptor and the activation of PLC with IP_3 production and IP_3R-mediated Ca^{2+} release from internal stores. Ca^{2+} release induced by testosterone also activates a Ras/MEK/ERK pathway, which is known to be involved in the extracellular response to growth factors that elicit hypertrophic processes in myocytes [46]. In cardiac myocytes, these effects were mimicked by cell-impermeant testosterone complexed to albumin (T-BSA), and were not inhibited by the intracellular AR antagonist cyproterone. This result suggest that a membrane-resident AR different from the classic AR mediates these responses. Interestingly, this Ca^{2+} signal was complex and demonstrated to contain at least 3 components [47]. First, in the presence of extracellular Ca^{2+}, cardiac myocytes stimulated with testosterone enhanced their basal Ca^{2+} levels *via* a mechanism that involves L-type Ca^{2+} channels. Second, the magnitude of the basal Ca^{2+} oscillations was enhanced by testosterone, in a pathway that involves crosstalk between L-type Ca^{2+} channels and RyRs. Third, in the absence of extracellular Ca^{2+}, cardiac myocytes evidenced a $G_{\beta\gamma}$/PLC/IP_3/IP_3R pathway that leads to a nuclear Ca^{2+} with kinetics faster than the cytosolic Ca^{2+} increases.

From these responses, multiple perspectives emerge. On one hand, the cardiomyocyte contraction process mediated by Ca^{2+}-induced Ca^{2+} release could be inotropically enhanced, as the basal oscillations were increased in magnitude but not in frequency. On the other hand, the increase in nuclear Ca^{2+} is likely to be involved in the modulation of gene expression. Further elucidation of these processes will certainly reveal new pathways in the hypertrophic response of cardiac myocytes to AAS. However, new insights have already been obtained regarding the relationship between cytosolic Ca^{2+} levels and the activation of the mammalian target of rapamycin (mTOR) complex 1, which is a master regulator of cell growth by transcriptional activity and protein synthesis [48]. In cultured cardiac myocytes, both the MEK/ERK pathway and the mTOR/S6K axis are stimulated by testosterone, leading to increased cell size, amino acid incorporation, and augmented expression of β-myosin heavy chain and αskeletal actin in an IP_3R/Ca^{2+}-dependent fashion [26]. These results lend yet more evidence to a model where Ca^{2+} serves as a novel central regulator of cardiac myocyte hypertrophy in response to AAS. Thus, nongenomic responses mediated by Ca^{2+} operate in concert with transcriptional responses, establishing a functional link between these processes which were previously thought to be independent (Fig. **2**). In concordance with these findings, the non-permeant T-BSA complex has been shown to promote actin cytoskeleton reorganization *via* nongenomic responses in prostate cancer cells [49]. Additionally, Ca^{2+} transients have been reported to increase the binding of testosterone to intracellular ARs [50]. Further studies will elucidate more details of the triangular interplay among androgens, Ca^{2+} and hypertrophy, in particular regarding the participation of classical hypertrophy-related transcription factors. With time, we expect these insights to be relevant to skeletal muscle cells as well.

2.4. Calcium Oscillations and Neuronal Apoptosis

The brain represents a hallmark target site for the action of steroidal hormones. Both adrenal and gonadal steroids play important roles in brain development and function *via* genomic and nongenomic mechanisms [1, 51-55]. In addition, neurons constitute an elegant and complex model for the study of Ca^{2+} signals, mainly because Ca^{2+} regulates essential neural processes including synaptic plasticity [56], exocytosis [57], gene expression [58, 59], bioenergetics [60], autophagy [61] and apoptosis [62]. It has been described that estrogen promotes survival of neurons [63]; and mitochondrial Ca^{2+} levels seem to participate in these responses *via* metabolic mechanisms [64]. In contrast, testosterone may exert opposite effects upon neuronal survival, for instance by decreasing neural function and the mass of dopaminergic neurons in animal models [65]. These findings are consistent with the neuronal loss observed on human AAS abusers [66], as well as with the augmented risk of developing psychiatric disorders among these individuals [67]. On the other hand, it has been described that high doses of AAS induce apoptotic cell death in cardiac myocytes [68]. Interestingly, testosterone-driven changes in intracellular Ca^{2+} levels are similar in cardiac myocytes and neurons, namely rapid increase in Ca^{2+} oscillations.

Ca^{2+} oscillations represent, in comparison to single Ca^{2+} transients, a more complex signal to decode by cells, basically because they contain additional information

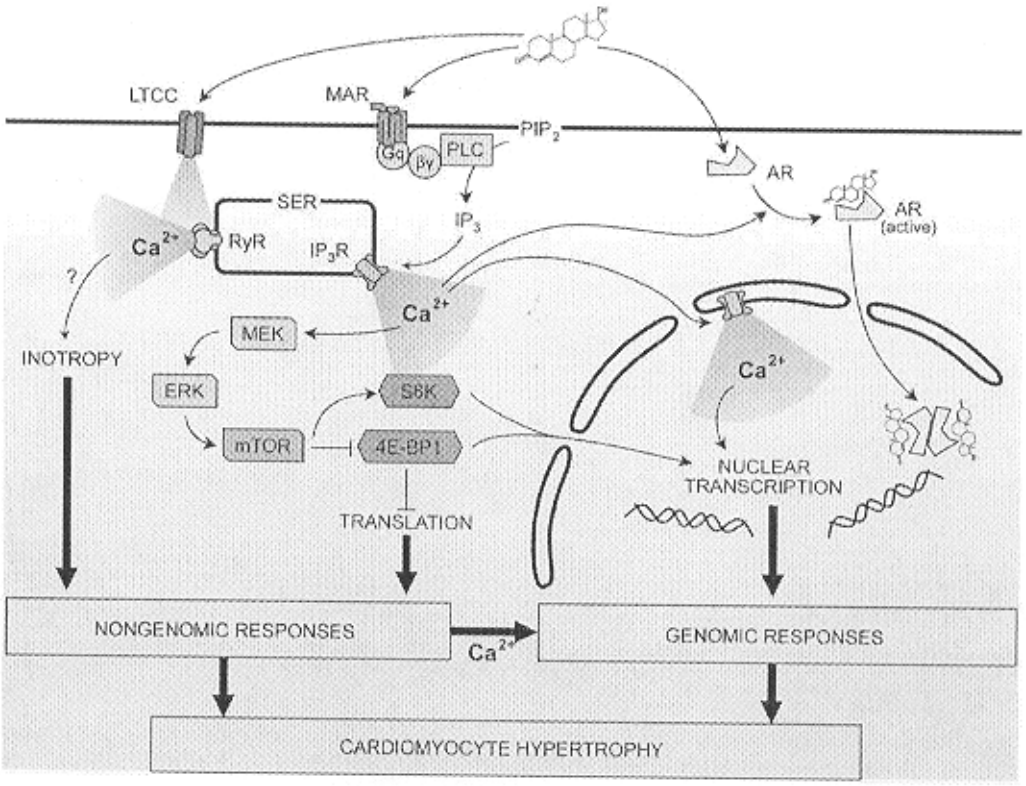

Fig. (2). Effects of AAS on intracellular calcium. In myotubes, as well as in cardiomyocytes, testosterone activates plasma membrane androgen receptors (MAR) coupled to heterotrimeric Gq protein. $G_{\beta\gamma}$ dimers activate phospholipase C (PLC), which locally produces inositol 1,4,5-trisphosphate (IP_3) thus activating IP_3 receptors (IP_3R). The consequent Ca^{2+} increase leads to the activation of several pathways, including the MEK/ERK/mTOR axis, propagation of nuclear Ca^{2+} signals and modulation of intracellular AR affinity for testosterone (an effect observed in platelets). These responses combined induce a series of genomic changes that lead to increased gene expression and increased protein synthesis, ultimately leading to cardiac hypertrophy. Although not studied in further detail, the effect at the left side of the figure upon Ca^{2+} release from ryanodine receptors (RyR), might lead to inotropy and therefore contribute *via* nongenomic changes to the hypertrophic phenotype. An overview of these processes is presented in the lower part of the figure, which illustrates how Ca^{2+} is centrally regulating the crosstalk between nongenomic and genomic responses.

encoded in their frequency and amplitude (for more details, please read [69]). These two parameters have been reported to activate transcriptional genomic responses [70, 71], mediate cardiac hypertrophy [72], or to enhance dendritic growth in neurons [73]. Transient testosterone addition to human neuroblastoma cells produces rapid intracellular Ca^{2+} oscillations that correlated with an increase in neurite growth. This response is dependent on concerted interactions between Ca^{2+} release through type-1 IP_3Rs and Ca^{2+} influx from the extracellular medium [74]. Importantly, these responses were obtained with 100 nM testosterone, a concentration that is on the high circulating range observed in humans, and therefore depends on physiological conditions such as gender, the physical state, age, or brain aromatase levels. This increase in neurite growth might be related to brain development and brain plasticity events observed with advanced age. However, higher testosterone levels are usually found among individuals exposed to testosterone replacement therapy or AAS abuse. In concordance with this notion, it has been observed that concentrations of 1-10 μM testosterone lead to higher and sustained Ca^{2+} signals that produce neuronal apoptosis [75]. This neuronal cell death cascade requires caspase activation and is inhibited by pharmacological inhibition or siRNA-mediated depletion of type-1 IP_3Rs. Therefore, a dual response is produced by testosterone on neuronal cells, on one hand promoting synaptic plasticity at physiological concentrations, and on the other hand, leading to apoptosis at higher concentrations (Fig. 3). Further details of these mechanisms are urgently needed to understand these effects in terms of the genetic responses involved, the possible adaptative mechanisms to chronic androgen administration and the clinical consequences in individuals exposed to high levels of androgens.

2.5. Circadian Regulation of AAS Signaling

There is a growing appreciation for the importance of circadian rhythms in human health and disease. These 24-hour cycles anticipate changes in physiological demand and help to coordinate appropriate responses or provide temporal separation of opposing but equally important processes. In the case of AAS, there is evidence of circadian control at many levels ranging from timing of hormone release and receptor availability, to circadian changes in intercellular

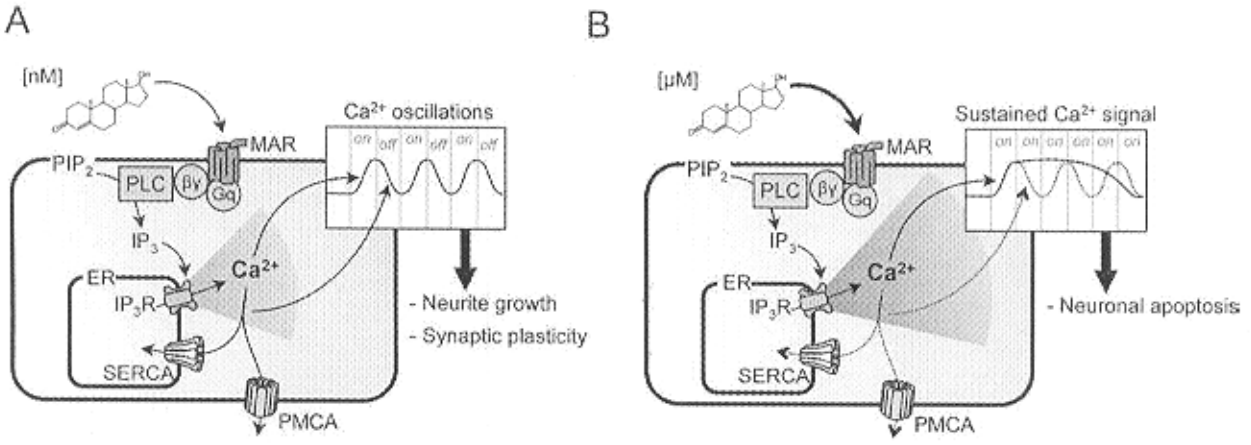

Fig. (3). Implications of Ca^{2+} oscillations in a neuronal model of AAS abuse. The study on the effects of testosterone upon neuroblastoma cells has provided two models for androgen action. (**A**) At physiological concentrations (nM), testosterone elicits intracellular Ca^{2+} oscillations, which are turned 'on' by inositol 1,4,5-trisphosphate receptor (IP_3R)-mediated Ca^{2+} release, and are turned 'off' by Ca^{2+} extruding mechanisms. These oscillations lead to an increase in neurite overgrowth, which might be related to synaptic plasticity. (**B**) At pathological concentrations (μM), testosterone persistence leads to enhanced activation of membrane androgen receptors (MAR), which lead to a sustained turning 'on' of the Ca^{2+} signal. Under these conditions, mitochondrial-dependent cascades of caspase activation lead to neuronal apoptosis.

Ca^{2+} levels. Conversely, testosterone can influence circadian day length and accordingly, sustained use of AAS has been associated with hyperactivity and disruption of circadian rhythm [76]. It is likely that this cross-regulation between AAS and circadian rhythms involves both genomic and nongenomic mechanisms of action, however, for the sake of this review we will touch briefly on the potential involvement of Ca^{2+} signaling. Cytoplasmic Ca^{2+} levels are circadian in a number of tissues including the suprachiasmatic nucleus (SCN), the region of the brain where the central clock mechanism resides, and Ca^{2+} acts as an important second messenger during clock resetting [77]. Immerging studies suggest that normally in the healthy heart, diastolic cytoplasmic Ca^{2+} levels are also circadian, which results in circadian activation of calcineurin and NFAT [78]. Cardiac myocytes require repair and maintenance, and this is most likely occurring during times of lower demand [79]. In mice, the peak observed in cardiac calcineurin/NFAT activity occurs at the beginning of the animal's resting phase and activation of this pathway is an important component of cardiac remodeling. Consistent with concept of cardiac rhythms in Ca^{2+} signaling, isolated adult cardiomyocytes display circadian variation in the magnitude of Ca^{2+} release in response to isoproterenol [80]. Similarly, both brain [81] and cardiac [82] tissue display diurnal rhythms in susceptibility to ischemic damage. As circulating levels of AAS are circadian, it is essential to consider their potential involvement in coordination of anabolic, catabolic, and cell survival cycles in order to fully understand their mechanism of action *in vivo*.

3. SUMMARY AND CONCLUDING REMARKS

Genomic responses of androgens, acting *via* binding to intracellular AR and changes in transcription, take hours to manifest, whereas a new set of rapid responses, termed 'nongenomic,' has been widely reported. Among the rapid effects of androgens, changes in intracellular Ca^{2+} represent a particularly powerful signaling mechanism, because Ca^{2+} is a central mediator of a diverse array of cell responses including entrainment and maintenance of circadian rhythmicity. In both skeletal muscle cells and cardiac muscle cells, testosterone elicits a rapid release of Ca^{2+} from IP_3-dependent and IP_3-independent stores, which are related to ERK signaling, mTOR signaling, gene expression and possible inotropic effects [26, 44, 47]. Together, these responses lead to myocyte hypertrophy, thus bridging nongenomic and genomic responses *via* established Ca^{2+}-dependent mechanisms. On the other hand, neurons are also a target for the action of steroidal hormones, and testosterone regulates several processes depending on its concentration. At physiological levels (nM), testosterone produces Ca^{2+} oscillations mediated by IP_3Rs and Ca^{2+} influx. These oscillations are responsible for enhancing neurite growth and thus might regulate synaptic plasticity during development or brain damage events. At pharmacological concentrations (μM), such as those found in individuals with androgen replacement therapy or AAS abuse, testosterone leads to prolonged and sustained Ca^{2+} transients, which unleash mitochondrial dependent caspase activation mechanisms and lead to neuronal apoptosis [74, 75]. With respect to the adverse cardiovascular consequences of chronic AAS treatment, these studies also provide new insights into the mechanisms responsible for increased blood pressure, increased risk of pathological cardiac hypertrophy, arrhythmias and stroke [24, 83]. Furthermore, due to the diversity of levels at which circadian-regulated processes can influence and cross-talk with AAS signaling, we propose that it will be essential to integrate circadian influences with genomic and nongenomic effects to fully understand the impact of AAS *in vivo*.

Thus far, intracellular Ca^{2+} signaling represents a significant and potentially new important effect of AAS in muscle cells, neurons and possibly many other cell types. Additional insights into the implication of these effects upon gene expression and long-term genomic responses are urgently needed, and are likely to reveal unexpected physiologically relevant links between nongenomic and genomic responses to androgenic steroids.

ACKNOWLEDGEMENTS

Acknowledgements and apologies are expressed to the scientists whose work was not cited here. This work was supported by FONDECYT [grant 1090276 to M.E. and grant 1080436 to S.L.], FONDAP [grant 15010006 to M.E., E.J. and S.L.], by the National Institutes of Health (to J.A.H. and B.A.R.), the American Heart Association (to M.I., J.A.H., and B.A.R.), the American Heart Association-Jon Holden DeHaan Foundation (to J.A.H.). R.B. is a Conicyt doctoral fellow. J.M.V. and A.E.F. hold a postdoctoral fellowship from Becas Chile and FONDECYT, respectively.

ABBREVIATIONS

AAS = anabolic androgenic steroids

AR = androgen receptor

Ca^{2+} = calcium

$[Ca^{2+}]$ = Ca^{2+} concentration

E-C = excitation-contraction

ER = endoplasmic reticulum

ERK = extracellular-regulated kinase

HDAC = histone deacetylase complex

IP_3 = inositol-1,4,5-trisphosphate

IP_3R = IP_3 receptor

LTCC = L-type Ca^{2+} channel

MAPK = mitogen-activated protein kinases

MAR = membrane AR

MCU = mitochondrial Ca^{2+} uniporter

mTOR = mammalian target of rapamycin

NCX = Na^+/Ca^{2+} exchanger

PIP_2 = phosphatidylinositol 4,5-bisphosphate

PLC = phospholipase C

PMCA = plasma membrane Ca^{2+} ATPase

RyR = ryanodine receptor

SERCA = sarco-endoplasmic reticulum Ca^{2+} ATPase

SR = sarcoplasmic reticulum

T-BSA = testosterone covalently bound to albumin

VDAC = voltage-dependent anionic channel

VDCC = voltage-dependent Ca^{2+} channel

SCN = suprachiasmatic nucleus

S6K = ribosomal protein S6 p70 kinase

4E-BP1 = translation initiation factor 4E binding protein 1

REFERENCES

[1] Michels, G.; Hoppe, U.C. Rapid actions of androgens. *Front. Neuroendocrinol.*, **2008**, *29*, 182-198.

[2] Lu, M.L.; Schneider, M.C.; Zheng, Y.; Zhang, X.; Richie, J.P. Caveolin-1 interacts with androgen receptor. A positive modulator of androgen receptor mediated transactivation. *J. Biol. Chem.*, **2001**, *276*, 13442-13451.

[3] Pi, M.; Parrill, A.L.; Quarles, L.D. GPRC6A mediates the non-genomic effects of steroids. *J. Biol. Chem.*, **2010**, *285*(51), 39953-64.

[4] Berridge, M.J.; Bootman, M.D.; Roderick, H.L. Calcium signalling: dynamics, homeostasis and remodelling. *Nat. Rev. Mol. Cell Biol.*, **2003**, *4*, 517-529.

[5] Vlasblom, R.; Muller, A.; Musters, R.J.; Zuidwijk, M.J.; Van Hardeveld, C.; Paulus, W.J.; Simonides, W.S. Contractile arrest reveals calcium-dependent stimulation of SERCA2a mRNA expression in cultured ventricular cardiomyocytes. *Cardiovasc. Res.*, **2004**, *63*, 537-544.

[6] Arguin, G.; Caron, A.Z.; Elkoreh, G.; Denault, J.B.; Guillemette, G. The transcription factors NFAT and CREB have different susceptibilities to the reduced Ca^{2+} responses caused by the knock down of inositol trisphosphate receptor in HEK 293A cells. *Cell Physiol. Biochem.*, **2010**, *26*, 629-640.

[7] Buvinic, S.; Almarza, G.; Bustamante, M.; Casas, M.; Lopez, J.; Riquelme, M.; Saez, J.C.; Huidobro-Toro, J.P.; Jaimovich, E. ATP released by electrical stimuli elicits calcium transients and gene expression in skeletal muscle. *J. Biol. Chem.*, **2009**, *284*, 34490-34505.

[8] Cardenas, C.; Liberona, J.L.; Molgo, J.; Colasante, C.; Mignery, G.A.; Jaimovich, E. Nuclear inositol 1,4,5-trisphosphate receptors regulate local Ca2+ transients and modulate cAMP response element binding protein phosphorylation. *J. Cell Sci.*, **2005**, *118*, 3131-3140.

[9] Eltit, J.M.; Garcia, A.A.; Hidalgo, J.; Liberona, J.L.; Chiong, M.; Lavandero, S.; Maldonado, E.; Jaimovich, E. Membrane electrical activity elicits inositol 1,4,5-trisphosphate-dependent slow Ca2+ signals through a Gbetagamma/phosphatidylinositol 3-kinase gamma pathway in skeletal myotubes. *J. Biol. Chem.*, **2006**, *281*, 12143-12154.

[10] Eisner, V.; Parra, V.; Lavandero, S.; Hidalgo, C.; Jaimovich, E. Mitochondria fine-tune the slow Ca(2+) transients induced by electrical stimulation of skeletal myotubes. *Cell Calcium.*, **2010**, *48*, 358-370.

[11] Casas, M.; Figueroa, R.; Jorquera, G.; Escobar, M.; Molgo, J.; Jaimovich, E. IP(3)-dependent, post-tetanic calcium transients induced by electrostimulation of adult skeletal muscle fibers. *J. Gen. Physiol.*, **2010**, *136*, 455-467.

[12] Er, F.; Gassanov, N.; Brandt, M. C.; Madershahian, N.; Hoppe, U.C. Impact of dihydrotestosterone on L-type calcium channels in human ventricular cardiomyocytes. *Endocr. Res.*, **2009**, *34*, 59-67.

[13] Er, F.; Michels, G.; Brandt, M.C.; Khan, I.; Haase, H.; Eicks, M.; Lindner, M.; Hoppe, U.C. Impact of testosterone on cardiac L-type calcium channels and Ca2+ sparks: acute actions antagonize chronic effects. *Cell Calcium.*, **2007**, *41*, 467-477.

[14] Hall, J.; Jones, R.D.; Jones, T.H.; Channer, K.S.; Peers, C. Selective inhibition of L-type Ca2− channels in A7r5 cells by physiological levels of testosterone. *Endocrinology.*, **2006**, *147*, 2675-2680.

[15] Scragg, J.L.; Dallas, M.L.; Peers, C. Molecular requirements for L-type Ca2− channel blockade by testosterone *Cell Calcium.*, **2007**, *42*, 11-15.

[16] Scragg, J.L.; Jones, R.D.; Channer, K.S.; Jones, T.H.; Peers, C. Testosterone is a potent inhibitor of L-type Ca(2+) channels. *Biochem. Biophys. Res. Commun.*, **2004**, *318*, 503-506.

[17] Wappl, E.; Mitterdorfer, J.; Glossmann, H.; Striessnig, J. Mechanism of dihydropyridine interaction with critical binding

residues of L-type Ca2+ channel alpha 1 subunits. *J. Biol. Chem.*, **2001**, *276*, 12730-12735.

[18] Alexandersen, P.; Haarbo, J.; Byrjalsen, I.; Lawaetz, H.; Christiansen, C. Natural androgens inhibit male atherosclerosis: a study in castrated, cholesterol-fed rabbits. *Circ. Res.*, **1999**, *84*, 813-819.

[19] English, K.M.; Jones, R.D.; Jones, T.H.; Morice, A.H.; Channer, K.S. Testosterone acts as a coronary vasodilator by a calcium antagonistic action. *J. Endocrinol. Invest.*, **2002**, *25*, 455-458.

[20] Rosano, G.M.; Leonardo, F.; Pagnotta, P.; Pelliccia, F.; Panina, G.; Cerquetani, E.; della Monica, P.L.; Bonfigli, B.; Volpe, M.; Chierchia, S. L. Acute anti-ischemic effect of testosterone in men with coronary artery disease. *Circulation*, **1999**, *99*, 1666-1670.

[21] Kapoor, D.; Goodwin, E.; Channer, K.S.; Jones, T.H. Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. *Eur. J. Endocrinol.*, **2006**, *154*, 899-906.

[22] Hanke, H.; Lenz, C.; Hess, B.; Spindler, K. D.; Weidemann, W. Effect of testosterone on plaque development and androgen receptor expression in the arterial vessel wall. *Circulation.*, **2001**, *103*, 1382-1385.

[23] Duell, P. B.; Bierman, E. L. The relationship between sex hormones and high-density lipoprotein cholesterol levels in healthy adult men. *Arch. Intern. Med.*, **1990**, *150*, 2317-2320.

[24] Baggish, A.L.; Weiner, R.B.; Kanayama, G.; Hudson, J.I.; Picard, M.H.; Hutter, A.M., Jr.; Pope, H.G., Jr. Long-term anabolic-androgenic steroid use is associated with left ventricular dysfunction. *Circ. Heart Fail.*, **2010**, *3*, 472-476.

[25] Basaria, S.; Coviello, A.D.; Travison, T.G.; Storer, T.W.; Farwell, W.R.; Jette, A.M.; Eder, R.; Tennstedt, S.; Ulloor, J.; Zhang, A.; Choong, K.; Lakshman, K.M.; Mazer, N.A.; Miciek, R.; Krasnoff, J.; Elmi, A.; Knapp, P.E.; Brooks, B.; Appleman, E.; Aggarwal, S.; Bhasin, G.; Hede-Brierley, L.; Bhatia, A.; Collins, L.; LeBrasseur, N.; Fiore, L.D.; Bhasin, S. Adverse events associated with testosterone administration. *N. Engl. J. Med.*, **2010**, *363*, 109-122.

[26] Altamirano, F.; Oyarce, C.; Silva, P.; Toyos, M.; Wilson, C.; Lavandero, S.; Uhlen, P.; Estrada, M. Testosterone induces cardiomyocyte hypertrophy through mammalian target of rapamycin complex 1 pathway. *J. Endocrinol.*, **2009**, *202*, 299-307.

[27] Schwarcz, M.D.; Frishman, W.H. Testosterone and coronary artery disease. *Cardiol. Rev.*, **2010**, *18*, 251-257.

[28] Schofield, R.S.; Hill, J.A.; McGinn, C.J.; Aranda, J.M. Hormone therapy in men and risk of cardiac allograft rejection. *J. Heart Lung Transplant.*, **2002**, *21*, 493-495.

[29] Bourghardt, J.; Wilhelmson, A.S.; Alexanderson, C.; De Gendt, K.; Verhoeven, G.; Krettek, A.; Ohlsson, C.; Tivesten, A. Androgen receptor-dependent and independent atheroprotection by testosterone in male mice. *Endocrinology*, **2010**, *151*, 5428-5437.

[30] Torkler, S.; Wallaschofski, H.; Baumeister, S.E.; Volzke, H.; Dorr, M.; Felix, S.; Rettig, R.; Nauck, M.; Haring, R. Inverse association between total testosterone concentrations, incident hypertension and blood pressure. *Aging Male.*, **2010**, In press.

[31] Naghi, J.J.; Philip, K.J.; Dilibero, D.; Willix, R.; Schwarz, E.R. Testosterone therapy: treatment of metabolic disturbances in heart failure. *J. Cardiovasc. Pharmacol. Ther.*, 2011, *16*(1): 14-23.

[32] Alves, M.L.; Gaffin, R.D.; Wolska, B.M. Rescue of familial cardiomyopathies by modifications at the level of sarcomere and Ca2− fluxes. *J. Mol. Cell Cardiol.*, **2010**, *48*, 834-842.

[33] Sakuma, K.; Yamaguchi, A. The functional role of calcineurin in hypertrophy, regeneration, and disorders of skeletal muscle. *J. Biomed. Biotechnol.*, **2010**, *2010*, 721219.

[34] Schulz, R.A.; Yutzey, K.E. Calcineurin signaling and NFAT activation in cardiovascular and skeletal muscle development. *Dev. Biol.*, **2004**, *266*, 1-16.

[35] van Empel, V.P.; De Windt, L.J. Myocyte hypertrophy and apoptosis: a balancing act. *Cardiovasc. Res.*, **2004**, *63*, 487-499.

[36] Song, K.; Backs, J.; McAnally, J.; Qi, X.; Gerard, R.D.; Richardson, J.A.; Hill, J.A.; Bassel-Duby, R.; Olson, E. N. The transcriptional coactivator CAMTA2 stimulates cardiac growth by opposing class II histone deacetylases. *Cell.*, **2006**, *125*, 453-466.

[37] Chiong, M.; Parra, V.; Eisner, V.; Ibarra, C.; Maldonado, C.; Criollo, A.; Bravo, R.; Quiroga, C.; Contreras, A.; Vicencio, J.M.; Cea, P.; Bucarey, J.L.; Molgo, J.; Jaimovich, E.; Hidalgo, C.; Kroemer, G.; Lavandero, S. Parallel activation of Ca(2+)-induced survival and death pathways in cardiomyocytes by sorbitol-induced hyperosmotic stress. *Apoptosis*, **2010**, *15*, 887-903.

[38] Xu, J.; Gong, N.L.; Bodi, I.; Aronow, B.J.; Backx, P.H.; Molkentin, J.D. Myocyte enhancer factors 2A and 2C induce dilated cardiomyopathy in transgenic mice. *J. Biol. Chem.*, **2006**, *281*, 9152-9162.

[39] Munoz, J.P.; Collao, A.; Chiong, M.; Maldonado, C.; Adasme, T.; Carrasco, L.; Ocaranza, P.; Bravo, R.; Gonzalez, L.; Diaz-Araya, G.; Hidalgo, C.; Lavandero, S. The transcription factor MEF2C mediates cardiomyocyte hypertrophy induced by IGF-1 signaling. *Biochem. Biophys. Res. Commun.*, **2009**, *388*, 155-160.

[40] Marsh, J.D.; Lehmann, M.H.; Ritchie, R.H.; Gwathmey, J.K.; Green, G.E.; Schiebinger, R.J. Androgen receptors mediate hypertrophy in cardiac myocytes. *Circulation.*, **1998**, *98*, 256-261.

[41] Wyce, A.; Bai, Y.; Nagpal, S.; Thompson, C.C. Research Resource: The androgen receptor modulates expression of genes with critical roles in muscle development and function. *Mol. Endocrinol.*, **2010**, *24*, 1665-1674.

[42] Roberts-Wilson, T.K.; Reddy, R.N.; Bailey, J.L.; Zheng, B.; Ordas, R.; Gooch, J.L.; Price, S.R. Calcineurin signaling and PGC-1alpha expression are suppressed during muscle atrophy due to diabetes. *Biochim. Biophys. Acta.*, **2010**, *1803*, 960-967.

[43] Frey, N.; McKinsey, T.A.; Olson, E.N. Decoding calcium signals involved in cardiac growth and function. *Nat. Med.*, **2000**, *6*, 1221-1227.

[44] Estrada, M.; Espinosa, A.; Muller, M.; Jaimovich, E. Testosterone stimulates intracellular calcium release and mitogen-activated protein kinases *via* a G protein-coupled receptor in skeletal muscle cells. *Endocrinology*, **2003**, *144*, 3586-3597.

[45] Estrada, M.; Liberona, J.L.; Miranda, M.; Jaimovich, E. Aldosterone- and testosterone-mediated intracellular calcium response in skeletal muscle cell cultures. *Am. J. Physiol. Endocrinol. Metab.*, **2000**, *279*, E132-139.

[46] Lorenz, K.; Schmitt, J. P.; Vidal, M.; Lohse, M.J. Cardiac hypertrophy: targeting Raf/MEK/ERK1/2-signaling. *Int. J. Biochem. Cell Biol.*, **2009**, *41*, 2351-2355.

[47] Vicencio, J.M.; Ibarra, C.; Estrada, M.; Chiong, M.; Soto, D.; Parra, V.; Diaz-Araya, G.; Jaimovich, E.; Lavandero, S. Testosterone induces an intracellular calcium increase by a nongenomic mechanism in cultured rat cardiac myocytes. *Endocrinology*, **2006**, *147*, 1386-1395.

[48] Hall, M.N. mTOR-what does it do? *Transplant. Proc.*, **2008**, *40*, S5-8.

[49] Papakonstanti, E.A.; Kampa, M.; Castanas, E.; Stournaras, C. A rapid, nongenomic, signaling pathway regulates the actin reorganization induced by activation of membrane testosterone receptors. *Mol. Endocrinol.*, **2003**, *17*, 870-881.

[50] Cabeza, M.; Flores, M.; Bratoeff, E.; de la Pena, A.; Mendez, E.; Ceballos, G. Intracellular Ca2− stimulates the binding to androgen receptors in platelets. *Steroids*, **2004**, *69*, 767-772.

[51] Remage-Healey, L.; Bass, A.H. Rapid, hierarchical modulation of vocal patterning by steroid hormones. *J. Neurosci.*, **2004**, *24*, 5892-5900.

[52] Evrard, H.C.; Balthazart, J. Rapid regulation of pain by estrogens synthesized in spinal dorsal horn neurons. *J. Neurosci.*, **2004**, *24*, 7225-7229.

[53] Balthazart, J.; Baillien, M.; Ball, G.F. Rapid and reversible inhibition of brain aromatase activity. *J. Neuroendocrinol.*, **2001**, *13*, 63-73.

[54] Park, S.Y.; Tournell, C.; Sinjoanu, R.C.; Ferreira, A. Caspase-3- and calpain-mediated tau cleavage are differentially prevented by estrogen and testosterone in beta-amyloid-treated hippocampal neurons. *Neuroscience*, **2007**, *144*, 119-127.

[55] Vasudevan, N.; Pfaff, D.W. Non-genomic actions of estrogens and their interaction with genomic actions in the brain. *Front. Neuroendocrinol.*, **2008**, *29*, 238-257.

[56] Lohmann, C. Calcium signaling and the development of specific neuronal connections. *Prog. Brain. Res.*, **2009**, *175*, 443-452.

[57] Pang, Z.P.; Sudhof, T. C. Cell biology of Ca2+-triggered exocytosis. *Curr. Opin. Cell Biol.*, **2010**, *22*, 496-505.

[58] Kemmerling, U.; Munoz, P.; Muller, M.; Sanchez, G.; Aylwin, M.L.; Klann, E.; Carrasco, M.A.; Hidalgo, C. Calcium release by ryanodine receptors mediates hydrogen peroxide-induced activation of ERK and CREB phosphorylation in N2a cells and hippocampal neurons. *Cell Calcium.*, **2007**, *41*, 491-502.

[59] Marchenko, S.M.; Thomas, R.C. Nuclear Ca2+ signalling in cerebellar Purkinje neurons. *Cerebellum.*, **2006**, *5*, 36-42.
[60] Abramov, A.Y.; Duchen, M.R. Impaired mitochondrial bioenergetics determines glutamate-induced delayed calcium deregulation in neurons. *Biochim. Biophys. Acta.*, **2010**, *1800*, 297-304.
[61] Vicencio, J.M.; Lavandero, S.; Szabadkai, G. Ca2+, autophagy and protein degradation: thrown off balance in neurodegenerative disease. *Cell Calcium.*, **2010**, *47*, 112-121.
[62] Harr, M.W.; Distelhorst, C.W. Apoptosis and autophagy: decoding calcium signals that mediate life or death. *Cold Spring Harb. Perspect. Biol.*, **2010**, *2*, a005579.
[63] Gould, E.; McEwen, B.S. Neuronal birth and death. *Curr. Opin. Neurobiol.*, **1993**, *3*, 676-682.
[64] Nilsen, J.; Brinton, R.D. Mitochondria as therapeutic targets of estrogen action in the central nervous system. *Curr. Drug Targets CNS. Neurol. Disord.*, **2004**, *3*, 297-313.
[65] Johnson, M.L.; Day, A.E.; Ho, C.C.; Walker, Q.D.; Francis, R.; Kuhn, C.M. Androgen decreases dopamine neurone survival in rat midbrain. *J. Neuroendocrinol.*, **2010**, *22*, 238-247.
[66] Petersson, A.; Garle, M.; Holmgren, P.; Druid, H.; Krantz, P.; Thiblin, I. Toxicological findings and manner of death in autopsied users of anabolic androgenic steroids. *Drug Alcohol Depend.*, **2006**, *81*, 241-249.
[67] Kanayama, G.; Hudson, J. I.; Pope, H. G., Jr. Long-term psychiatric and medical consequences of anabolic-androgenic steroid abuse: a looming public health concern? *Drug Alcohol Depend.*, **2008**, *98*, 1-12.
[68] Zaugg, M.; Jamali, N.Z.; Lucchinetti, E.; Xu, W.; Alam, M.; Shafiq, S.A.; Siddiqui, M.A. Anabolic-androgenic steroids induce apoptotic cell death in adult rat ventricular myocytes. *J. Cell Physiol.*, **2001**, *187*, 90-95.
[69] Uhlen, P.; Fritz, N. Biochemistry of calcium oscillations. *Biochem. Biophys. Res. Commun.*, **2010**, *396*, 28-32.
[70] Dolmetsch, R.E.; Xu, K.; Lewis, R.S. Calcium oscillations increase the efficiency and specificity of gene expression. *Nature*, **1998**, *392*, 933-936.
[71] Li, W.; Llopis, J.; Whitney, M.; Zlokarnik, G.; Tsien, R.Y. Cell-permeant caged InsP3 ester shows that Ca2+ spike frequency can optimize gene expression. *Nature*, **1998**, *392*, 936-941.
[72] Colella, M.; Grisan, F.; Robert, V.; Turner, J.D.; Thomas, A.P.; Pozzan, T. Ca2+ oscillation frequency decoding in cardiac cell hypertrophy: role of calcineurin/NFAT as Ca2+ signal integrators. *Proc. Natl. Acad. Sci. USA*, **2008**, *105*, 2859-2864.
[73] Desfrere, L.; Karlsson, M.; Hiyoshi, H.; Malmersjo, S.; Nanou, E.; Estrada, M.; Miyakawa, A.; Lagercrantz, H.; El Manira, A.; Lal, M.; Uhlen, P. Na,K-ATPase signal transduction triggers CREB activation and dendritic growth. *Proc. Natl. Acad. Sci. USA.*, **2009**, *106*, 2212-2217.
[74] Estrada, M.; Uhlen, P.; Ehrlich, B.E. Ca2+ oscillations induced by testosterone enhance neurite outgrowth. *J. Cell Sci.*, **2006**, *119*, 733-743.
[75] Estrada, M.; Varshney, A.; Ehrlich, B.E. Elevated testosterone induces apoptosis in neuronal cells. *J. Biol. Chem.*, **2006**, *281*, 25492-25501.
[76] Hayes, L.D.; Bickerstaff, G.F.; Baker, J.S. Interactions of cortisol, testosterone, and resistance training: influence of circadian rhythms. *Chronobiol. Int.*, **2010**, *27*, 675-705.
[77] Ikeda, M. Calcium dynamics and circadian rhythms in suprachiasmatic nucleus neurons. *Neuroscientist.*, **2004**, *10*, 315-324.
[78] Sachan, N.; Dey, A.; Rotter, D.; Grinsfelder, D.B.; Battiprolu, P.K.; Sikder, D.; Copeland, V.; Oh, M.; Bush, E.; Shelton, J.M.; Bibb, J.A.; Hill, J.A.; Rothermel, B.A. Sustained hemodynamic stress disrupts normal circadian rhythms in calcineurin-dependent signaling and protein phosphorylation in the heart. *Circ. Res.*, **2011**, *108*, 437-445.
[79] Boateng, S.Y.; Goldspink, P.H. Assembly and maintenance of the sarcomere night and day *Cardiovasc. Res.*, **2008**, *77*, 667-675.
[80] Collins, H.E.; Rodrigo, G.C. Inotropic response of cardiac ventricular myocytes to beta-adrenergic stimulation with isoproterenol exhibits diurnal variation: involvement of nitric oxide. *Circ. Res.*, **2010**, *106*, 1244-1252.
[81] Weil, Z.M.; Karelina, K.; Su, A.J.; Barker, J.M.; Norman, G.J.; Zhang, N.; Devries, A.C.; Nelson, R.J. Time-of-day determines neuronal damage and mortality after cardiac arrest. *Neurobiol. Dis.*, **2009**, *36*, 352-360.
[82] Durgan, D.J.; Pulinilkunnil, T.; Villegas-Montoya, C.; Garvey, M.E.; Frangogiannis, N.G.; Michael, L.H.; Chow, C.W.; Dyck, J.R.; Young, M.E. Short communication: ischemia/reperfusion tolerance is time-of-day-dependent: mediation by the cardiomyocyte circadian clock. *Circ. Res.*, **2010**, *106*, 546-550.
[83] Achar, S.; Rostamian, A.; Narayan, S.M. Cardiac and metabolic effects of anabolic-androgenic steroid abuse on lipids, blood pressure, left ventricular dimensions, and rhythm. *Am. J. Cardiol.*, **2010**, *106*, 893-901.

Received: December 22, 2010 Revised: January 21, 2011 Accepted: February 07, 2011