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The interdependence of Endothelin-1 and Calcium: A Review

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Abstract

The 21 amino acid peptide endothelin-1 (ET-1) regulates a diverse array of physiological processes, including vasoconstriction, angiogenesis, nociception, and cell proliferation. Most of the effects of ET-1 are associated with an increase in intracellular calcium concentration. The calcium influx and mobilization pathways activated by ET-1, however, vary immensely. This review will begin with the basics of calcium signaling, and investigate the different ways intracellular calcium concentration can increase in response to a stimulus. The focus will then shift to ET-1, and discuss how ET receptors mobilize calcium. We will also examine how disease alters calcium-dependent responses to ET-1 by discussing changes to ET-1-mediated calcium signaling in hypertension, since there is significant interest in the role of ET-1 in this important disease. A list of unanswered questions regarding ET-mediated calcium signals are also presented, as well as perspectives for future research of calcium mobilization by ET-1.

Keywords

endothelin; calcium signaling; G protein-coupled receptors; ion channels; vasculature

Introduction

Endothelin-1 (ET-1) is a 21-amino acid peptide, originally characterized as an endothelium-derived constricting factor in the vasculature [1, 2]. ET-1 also affects a host of non-vascular tissues, including brain, kidney, intestine and adrenal gland [3]. Further experimentation discovered two G protein-coupled receptors (GPCR's) to which ET-1 binds: the ET_A and ET_B receptor. Physiological responses to ET-1 can be attributed to ET_A receptors, ET_B receptors, or both. ET-1 is not solely a vasoconstrictor; ET-1 stimulates angiogenesis, induces astrocyte proliferation, activates nociceptive neurons, constricts bronchi, and stimulates the production of several inflammatory mediators in neutrophils and macrophages [4-7]. Dysfunction or dysregulation in the endothelin (ET) system is present in chronic pain, acute renal failure, asthma, colorectal cancer, and stroke, but dysfunction is most apparent in vascular diseases like hypertension [8-11].

The role of ET-1 in the vasculature extends beyond its vasoconstricting properties. Plasma ET-1 levels are elevated in humans with salt-sensitive essential hypertension, and vascular ET-1 expression is increased in severe hypertension [12]. In types of human hypertension where plasma ET-1 remains steady, vascular tissues from hypertensive humans exhibit exaggerated reactivity to ET-1 [13]. In the DOCA-salt model of hypertension, vascular contraction to ET-1 is decreased even though plasma ET-1 concentrations are increased [14]. This implies that the tissue responses to ET-1 are altered due to dysfunctional ET

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receptor signaling and not concentration-dependent activation of ET-1-mediated pathways [15].

As the number of biological responses affected by ET-1 grows, one tenet remains unchanged: many, if not most, of the responses to ET-1 are calcium-dependent. Whether ET receptors increase intracellular calcium concentration ($[Ca^{2+}]_i$) by activating extracellular calcium influx or intracellular calcium release depends on the tissue, ET receptor type and the response being measured [16-18]. These increases in $[Ca^{2+}]_i$ can be due to voltage-dependent calcium influx, store-operated calcium entry, voltage-independent calcium influx, release of one of several intracellular calcium stores, or any combination therein [19-21]. The calcium increases in some cells are transient; in others, ET-1 causes a slow and prolonged increase in intracellular calcium. Subtle changes to these calcium currents can cause major alterations in cellular function, ultimately leading to the pathogenesis of disease. As such, the complex mechanisms by which ET-1 can modulate intracellular calcium to alter cellular function remain a novel and intriguing area of investigation, and are the focus of this review. While this review will discuss mechanisms common to ET-1-dependent responses in many tissue types and diseases, the effects of ET-1 in the vasculature during hypertension are highlighted.

This review will begin with a primer on calcium signaling, regulation of calcium influx, and mobilization of calcium form intracellular calcium stores. We will then explore how calcium influx and mobilization are activated by ET-1, and how the interactions between calcium and ET-1 are altered during hypertension. Finally, we will present a list of unanswered questions regarding ET-1-mediated calcium signaling, and offer our perspectives for future research of calcium mobilization by ET-1.

The Basics of Calcium Signaling

Responses regulated by ET-1 have been associated with increases in $[Ca^{2+}]_i$, either by influx of calcium or release of intracellular calcium stores. $[Ca^{2+}]_i$ is tightly regulated by a multitude of ion channels and exchangers that control influx, efflux, sequestration, and release of calcium [22-24]. Table 1 outlines the different types of plasma membrane calcium channels, and the receptors that modulate intracellular calcium release. Included is a description of their characteristics, known pharmacological activators, and known pharmacological inhibitors.[19, 25-31]

Increases in $[Ca^{2+}]_i$ can be due to influx only, stores release only, or a portion of both – and the contribution of each source of calcium varies between receptors. This complex regulatory mechanism exists to control $[Ca^{2+}]_i$ because small changes in amplitude, duration and location of calcium influx are sufficient to cause a wide variation of physiological responses [32]. The pathways for calcium influx and calcium stores release are multi-faceted and tightly controlled, since small changes in intracellular calcium can be the difference between cell survival and cell death [33]. Before examining how ET-1 can increase $[Ca^{2+}]_i$, we will briefly discuss the mechanisms utilized by many GPCR's to increase calcium influx and cause $[Ca^{2+}]_i$ release.

Calcium Influx—Generally, calcium enters a cell by passing through a calcium channel that opens in response to any number of stimuli. The calcium concentration within a cell is much lower than the calcium concentration in the extracellular fluid (100 nM *vs.* 2.5 mM, respectively) [34]. This calcium concentration gradient allows calcium ions to move through the channels and into a cell by passive diffusion. Membrane depolarization, ligand binding, and release of intracellular stores are all capable of causing plasma membrane calcium channels to open [35]. Those that open due to membrane depolarization are the voltage-gated calcium channels (VGCC's) and any others are considered voltage-independent

calcium channels (VICC's). The VICC's can be further broken down into store-operated calcium channels (SOCC's), ligand-gated calcium channels (LGCC's) and non-selective cation channels (NSCC's).

Release of Calcium Stores—The major store of intracellular calcium is the endoplasmic reticulum, or the sarcoplasmic reticulum in muscle cells [23]. Calcium is liberated from sarcoplasmic/endoplasmic reticulum (SER) stores through two calcium channels: inositol 1,4,5-trisphosphate (IP_3) receptors and ryanodine receptors [36, 37].

IP_3 is produced when phospholipase C (PLC) hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2), generating both IP_3 and diacylglycerol (DAG) [38]. DAG affects calcium stores release indirectly, while IP_3 does so directly. DAG activates protein kinase-C (PKC), which then can inhibit IP_3 production by PLC [39]. PKC also phosphorylates VGCC's, which alters their function to either inhibit or sustain calcium influx [21]. IP_3 activates IP_3 receptors on the SER membrane, which then open and allow Ca^{2+} to leave the SER and enter the cytoplasm [40].

Ryanodine receptors, when activated by local increases in intracellular calcium, cause additional calcium release from SER stores [41]. As such, ryanodine receptors amplify small calcium signals caused by voltage-dependent calcium influx or IP_3-mediated calcium release [42]. In addition to amplification of calcium signals, ryanodine receptors are involved in the termination of calcium influx across the plasma membrane. Ryanodine receptors are on the SER membrane closest to the plasma membrane, whereby a "spark" of calcium from ryanodine receptors will activate calcium-sensitive potassium channels and close VGCC's as the membrane hyperpolarizes [43]. Ryanodine receptors serve to amplify calcium signals rapidly, but then to also terminate voltage-dependent calcium influx.

Thus, IP_3 receptors and ryanodine receptors activate pathways that tightly regulate Ca^{2+} release from SER stores, and regulate voltage-dependent Ca^{2+} entry both spatially and temporally. The interplay between PLC, IP_3, DAG, and PKC also keeps intracellular Ca^{2+} concentration precisely controlled, while still allowing for rapid release of minute amounts of Ca^{2+} in response to a stimulus.

The Relationship between ET-1 and Calcium

The interdependence between ET-1 and calcium is apparent when examining both the physiological effects of ET receptor activation and ET-1 synthesis. Although multiple cell types synthesize ET-1, the predominant sources of ET-1 are vascular endothelial cells [44]. Molecules that increase endothelial cell $[Ca^{+2}]_i$ augment expression of preproendothelin-1 (ppET-1) mRNA *via* a calcium/calmodulin/calmodulin kinase (Ca^{2+}/CaM/CaM-K) pathway [45, 46]. The physiological responses elicited by ET-1 can be both calcium-dependent and calcium-independent [16, 47, 48]. Some examples of calcium-dependent processes regulated by ET-1 can be found in Table 2. Table 2 also separates ET_A receptor-dependent responses from ET_B receptor responses, and notes the specific calcium sources activated by each receptor. In some responses, ET_A receptors regulate calcium store release and ET_B receptors regulate calcium influx (*e.g.* bronchoconstriction). In others, ET_A or ET_B receptors regulate both calcium influx and stores release. No correlation exists between ET receptor subtype and the source of calcium governing the response. Thus, calcium influx and mobilization by ET-1 is cell type-specific, with regard to which ET receptor subtypes are involved. [49-64]

Some of the most-studied effects of ET-1 are those in the vasculature, where ET-1 acts as a potent vasoconstrictor [65]. ET-1-induced increases in $[Ca^{+2}]_i$ are similar in pattern to those caused by other calcium-dependent vasoconstrictors, where calcium increases in two stages. First, there is an initial increase in $[Ca^{+2}]_i$ from intracellular calcium stores, which is

followed by a sustained increase in $[Ca^{+2}]_i$ due to the influx of calcium from the extracellular space [20]. This pattern of initial release/sustained influx is evident in non-vascular cells as well, whereby ET-1 can induce neutrophil migration, attenuate cGMP formation in astrocytes, stimulate diuresis and natriuresis, and cause vasorelaxation [56, 66-68]. What differs between cells and tissues are the *specific* mechanisms that regulate the initial extracellular calcium influx and the sustained intracellular calcium mobilization in response to ET receptor activation.

Cytoplasmic Calcium Changes Evoked by ET-1

Voltage-Dependent Calcium Influx

Mechanism—The specific VGCC's implicated in ET-1-induced calcium entry vary, which is not surprising due to the range of responses ET-1 influences (*see* Figure 1). L-type, T-type, and R-type calcium channels have all been associated with voltage-dependent calcium influx caused by ET-1, and the relative involvement of each channel type depends on the species and cell type being studied [69, 70]. The activation of multiple calcium channels during voltage-dependent calcium influx is not unique to ET-1; what is interesting is that ET-1 may regulate VGCC's directly as well as indirectly. The idea that ET-1 can act as a calcium channel opener was proposed as early as 1988, but it is also possible that ET receptor activation alters the voltage-gating properties of VGCC's indirectly, to increase calcium influx through them [71, 72]. Several researchers published compelling evidence against the theory that ET-1 was a direct agonist of L-type VGCC's, instead postulating that ET-1 altered VGCC function through second messengers like PLC and protein kinase C (PKC) [73-75].

ET Receptor Dependence—Voltage-dependent calcium entry is regulated by neither the ET_A receptor nor the ET_B receptor exclusively. Depending on the cell, tissue, and experimental conditions, activation of either or both ET receptors can regulate voltage-dependent calcium influx. In cardiac myocytes, for example, ET_A receptors as well as ET_B receptors regulate specific voltage-dependent calcium currents [76]. ET_A receptors mediate ET-1-dependent inhibition of voltage-dependent calcium currents caused by isoproterenol. In the same cells, ET-1-dependent stimulation of calcium currents after exposure to atrial natriuretic peptide (ANP) is mediated by ET_B receptors. Cell, tissue, and conditional variability has made it difficult to characterize the exact mechanisms by which each ET receptor causes voltage-dependent calcium influx, as well as the relative contribution and importance of voltage-dependent calcium influx to ET-1-mediated responses. Nevertheless, ET receptor-mediated membrane depolarization and voltage-dependent calcium influx is an important mechanism by which ET-1 can increase $[Ca^{+2}]_i$ [77-79].

Changes in Hypertension—Pharmacological inhibition of voltage-dependent calcium influx is a well-established and often-used treatment for hypertension, as calcium channel blockers (*e.g.* nifedipine) decrease blood pressure by inhibiting calcium influx and reducing vasoconstriction [80]. These drugs have cardio-protective benefits as well; prolonged treatment with nifedipine not only lowers blood pressure, but it also improves endothelium-dependent vasorelaxation and reduces ET-1-dependent contraction [81]. Another calcium channel blocker, lacidipine, decreases ventricular hypertrophy and prepro-ET-1 expression in spontaneously-hypertensive rats [82]. These findings reinforce the importance of calcium mobilization in the vasculature, and show that the relationship between ET-1 and calcium is not a one-way street: ET-1 mobilizes calcium, but increases in calcium also augment the synthesis of ET-1. So, inhibition of voltage-dependent calcium influx decreases ET-1's deleterious effects on vascular function during hypertension, while simultaneously decreasing transcription of ET-1 precursors and ultimately reducing ET-1 production.

Voltage-Independent Calcium Influx

Mechanism—Regardless of the type of VGCC's associated with ET-1-induced calcium influx or the direct/indirect activation of VGCC's by ET-1, inhibition of all voltage-dependent calcium channels does not abolish the inward calcium currents caused by ET-1 [83]. In some excitable tissues, VGCC's are not activated in response to ET-1 [84]. Therefore, the remaining ET-1-induced calcium influx is through any of several voltage-independent calcium entry pathways (*see* Figure 1). As previously defined, voltage-independent calcium channels (VICC's) include calcium channels that are activated by a ligand directly (LGCC's), activated by intracellular calcium release (SOCC's), or by indirect activation through G-protein-dependent signaling pathways (NSCC's) (*see* Table 1). Although no LGCC activated by ET-1 is known currently, pharmacological investigation shows SOCC's and NSCC's are important influx pathways in ET-1-induced smooth muscle contraction, MAP Kinase phosphorylation, and arachidonic acid release [85-87]. The relative contribution of SOCC's and NSCC's to ET-1-mediated calcium influx is dependent on the concentrations of ET-1 used. Inhibition of NSCC's or SOCC's by SKF-96365 or LOE-908, respectively, abolished the calcium currents caused by low concentrations of ET-1 (≤ 0.1 nM) [88]. In the same study, however, calcium currents caused by higher concentrations of ET-1 were only abolished by a combination of SKF-96365 and LOE-908. Since the opening of both SOCC's and NSCC's could be stimulated by release of intracellular calcium stores, differentiating ET-1-induced extracellular calcium influx from calcium-induced calcium influx has proven difficult [29].

ET Receptor Dependence—Similar to voltage-dependent calcium entry, voltage-independent calcium entry can be regulated by either ET_A receptors or ET_B receptors, depending on the cell or tissue type [85]. Neither ET receptor is associated with calcium influx through only one type of VICC in all cell types.

Changes in Hypertension—Changes to voltage-independent calcium entry in hypertension are not well described, but calcium influx through VICC's appears to have little affect on systemic blood pressure. Treatment with Ginoside-Rd, a purported VICC inhibitor, did not lower systemic blood pressure in hypertensive rats [89, 90]. In the same experiment, however, VICC inhibition decreased vascular remodeling and ET-1-induced smooth muscle cell proliferation [90]. So, while there is little evidence that voltage-independent calcium influx is involved in the pathogenesis of hypertension, both ET-1 and VICC's are implicated in the progression of hypertension-induced vascular hypertrophy.

Release of Intracellular Calcium Stores

Mechanism—ET receptors cause intracellular calcium stores release by activating PLC and increasing IP_3 production [20, 91]. Similar to ET-1's effects on calcium influx pathways, the calcium released from IP_3-sensitive stores does not account for the increase in $[Ca^{+2}]_i$ entirely [21]. Intracellular calcium must also come from other reticular stores (*e.g.* ryanodine-sensitive stores) or an atypical intracellular calcium store (*e.g.* mitochondrial stores and lysosomal stores) that ET-1 can mobilize. In peritubular smooth muscle cells and renal afferent arterioles, ET-1 alters cyclic-ADP ribose production to sensitize ryanodine-activated SER stores [92-94]. Neither IP_3-sensitive stores nor atypical calcium stores, however, account for ET-1-induced increases in intracellular calcium entirely, consistently, and across cell types.

ET Receptor Dependence—While many studies confirm that ET-1 causes intracellular calcium release, none provide evidence that ET_A receptors and ET_B receptors mobilize different intracellular calcium stores. The intracellular calcium stores mobilized by ET-1 depend upon the cell type, and not the ET receptor subtype.

Changes in Hypertension—Smooth muscle cells from hypertensive rats maintain increased $[Ca^{2+}]_i$ after depolarization, which implies intracellular calcium storage and mobilization are altered during hypertension [95]. However, IP_3-mediated calcium released by ET-1 stimulation is blunted in DOCA-salt hypertension and unchanged in spontaneously-hypertensive rats [96]. Thus, even though basal $[Ca^{2+}]_i$ is increased in hypertension, the ability of ET-1 to mobilize calcium directly is impaired.

The Possibilities: Beyond Calcium Channels and Reticular Stores

Increases in intracellular calcium caused by ET-1 are due to a mixture of voltage-dependent calcium influx, voltage-independent calcium influx, and mobilization of calcium from multiple intracellular stores. These mechanisms vary in their predominance as a calcium source at different times, in different cell types, and at different concentrations of ET-1. No calcium source is linked to ET_A or ET_B receptors with any specificity across multiple cell types or tissues, since either ET receptor can cause calcium influx through identical pathways in different tissues. ET-1-mediated responses utilize a combination of all of these calcium sources, and no one source dominates across multiple cell types. While many of these individual pathways have been investigated, it is still unclear how ET-1 modulates both influx and release of calcium simultaneously, and these mechanisms do not account for all the calcium that is mobilized in response to ET-1.

Recent exploration into ET-1's calcium signaling pathways has offered some interesting alternatives to the traditional influx/release pathways attributed to ET-1-induced calcium mobilization, which will be discussed next. While these novel findings are not able to integrate and explain the entirety of ET-1-induced calcium mobilization, they present new and different insights into the function of ET-1 and ET receptors, as well as novel mechanisms for maintaining intracellular calcium concentrations.

Calcium Extrusion

In addition to calcium influx mechanisms that increase $[Ca^{2+}]_i$, there are calcium efflux mechanisms that lower the concentration of intracellular calcium back to basal levels. Due to the large inward concentration gradient, calcium efflux requires the use of energy (in the form of ATP) to push calcium out of the cell and back into the extracellular space [34]. If these mechanisms were inhibited by ET-1, the net result would be prolonged elevations of intracellular calcium concentration due to retention of calcium [97]. Thus, inhibition of calcium efflux mechanisms represents another means by which ET-1 can modify $[Ca^{2+}]_i$. ET-1 suppresses plasma membrane Calcium-ATPase function and expression in hepatic sinusoidal endothelial fenestrae, leading to contraction [98]. ET-1 also causes the sodium/calcium exchanger (NCX) in the plasma membrane of ventricular myocytes to operate in reverse mode, whereby NCX become calcium influx pumps instead of acting as calcium efflux pumps [99]. Future research is needed to elucidate the physiological effects which can be attributed to prolonged increases in $[Ca^{2+}]_i$ by ET-1.

ET-1 Signaling in the Nucleus

Traditionally, ET receptors are thought to be plasma membrane receptors, where they can be activated by extracellular ET-1 to initiate a G-protein-dependent intracellular signaling cascade. Recent work by Bkaily *et al*, shows the presence of ET_B receptors and R-type VGCC's in the nuclear membrane (*see* Figure 1). They further postulate that internalization of plasma-membrane ET receptors frees ET-1 from the receptor, and this cytosolic ET-1 activates ET_B receptors in the nuclear membrane [100]. The activation of nuclear ET_B receptors causes an increase in nuclear calcium by opening R-type calcium channels and Na^+/Ca^{2+} exchangers (NCX) on the nuclear membrane, as well as indirect activation of IP_3

receptors and ryanodine receptors located in the nucleoplasmic reticulum [101]. The sequestration of calcium within the nucleus may serve as a regulatory element for maintaining calcium homeostasis within the cell, much like sarcoplasmic reticular or mitochondrial uptake of calcium. It also may regulate the expression of calcium-sensitive genes, or act as a protective mechanism to buffer the nucleus against calcium depletion or overload [102, 103]. If calcium can enter the nucleus, it is likely that it can leave the nucleus as well. What remains to be seen is if nuclear ET receptors signal differently than membrane ET receptors, and how intracellular ET-1 can regulate both intra-nuclear and intracellular calcium mobilization.

Understanding of the function of nuclear GPCR's is a work-in-progress. Much of this research has focused on the angiotensin (AT) AT_1 receptor. Binding sites for angiotensin-II on the nuclei of rat hepatocytes turned out to be AT_1 receptors that regulate reactive oxygen species (ROS) production [104, 105]. Further investigation will explain the function of nuclear ET receptors and their role in maintaining calcium homeostasis.

ET Receptor Dimerization

GPCR's are capable of forming homodimers (*e.g.* 2 β_2 adrenergic receptors together) and heterodimers (*e.g.* M_2 muscarinic receptor with an M_3 muscarinic receptor) [106, 107]. Research into GPCR dimerization is increasing as the physiological relevance of GPCR dimers becomes more apparent [108]. HEK-293 cells transfected with human ET_A receptors, human ET_B receptors, or both ET_A and ET_B receptors show that these receptors can form both homodimers (ET_A/ET_A; ET_B/ET_B) and heterodimers (ET_A/ET_B) [109]. The physiological and pharmacological evidence of ET receptor dimers continues to grow as well, as ET_A and ET_B receptors functionally interact in human bronchi, saphenous vein, and C6 glioma cells [110-114]. ET receptors have the N- and C-terminal binding motifs for covalent interaction, and can be successfully co-immunoprecipitated from transfected cells [115]. While co-immunoprecipitation of ET_A and ET_B receptors from many native tissues has proven difficult, the ET receptors have been co-precipitated successfully rat pulmonary resistance arteries [116].

When the transfected HEK-293 cells were used to investigate the effects of ET receptor dimers on ET-1-induced calcium signaling, ET_A and ET_B homodimers mediated transient increases in $[Ca^{+2}]_i$ (approximately 1 minute), while $ET_{A/B}$ heterodimers caused a sustained increase in $[Ca^{+2}]_i$ that lasted over 10 minutes [117, 118]. Thus, the presence of different ET receptor homo- and heterodimers changes the profile of the calcium response. These studies did not examine the mechanisms by which dimers alter calcium signaling, but the fact that dimers can modify calcium signaling is fascinating. Exploring how ET receptor dimers can change calcium influx, alter release of intracellular calcium, or decrease calcium extrusion and sequestration is worth further investigation.

Perspectives

Where do we go from here, as we continue to disassemble the mechanisms by which ET-1 increases intracellular calcium? Below are thoughts on this question, where we consider mechanisms that may become relevant to ET-1-dependent calcium signaling as research progresses.

ET Receptors on other Organelles

The presence of receptors and ion channels on intracellular organelles is not limited to the nucleus, as functional TRPV1 channels were discovered on the endoplasmic reticular membrane of dorsal root ganglion neurons [119]. If ET receptors can be found in the

membrane of calcium-storing organelles, such as mitochondria or endoplasmic reticulae, they may mobilize calcium and alter cellular function in new and novel ways. If this were true, such a discovery would change the way we think about both calcium signaling and GPCR function.

Receptor/Channel Interaction

The ability of ET receptors to alter the activity of other receptors, and *vice versa*, is already noted in the kidney, where ET_B receptors in the renal proximal tubule can interact with dopamine D_3 receptors (another GPCR) to alter natriuresis [120]. Interactions between different GPCR's can influence the type and function of ion channels associated with them, possibly by recruiting different scaffolding and accessory proteins to form a multi-meric signaling complex [121]. For example: TRPC1 receptors and BK channels can associate in vascular smooth muscle, and blockade of either TRPC1 or BK channels enhances ET-1 contraction of small mesenteric arteries [122]. Understanding if ET-1 can influence receptor and channel interactions remains relatively unexplored.

The Future: Calcium and Beyond

As seen in Figure 1, the current model of ET-1-dependent calcium mobilization is extremely complex for a single peptide with only two officially recognized receptors [3]. Equally numerous are the tools employed to elucidate the mechanisms, from *in vivo* experiments and *in vitro* functional assays to cell culture and cell transfection. As each piece of the puzzle of how ET-1 changes intracellular calcium is put into place, we see that it is part of a larger puzzle that challenges how we think about receptor-dependent calcium signaling mechanisms.

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List of Abbreviations

AT	angiotensin
AT_1	angiotensin II receptor type 1
BK channel	large-conductance calcium-activated potassium channel
Ca^{2+}/CaM/CaMK	calcium/calmodulin/calmodulin kinase
cGMP	cyclic guanosine monophosphate
DAG	diacylglycerol
ET-1	endothelin-1
GPCR	G protein-coupled receptor
IP_3	inositol trisphosphate
LGCC	ligand-gated calcium channel
MAPK	mitogen-activated protein kinase
NSCC	nonselective cation channel
PIP_2	phosphatidylinositol 4,5-bisphosphate
PKC	protein kinase C

PLC	phospholipase-C
ppET-1	preproendothelin-1
ROS	reactive oxygen species
RyR	ryanodine receptor
SER	sarcoplasmic/endoplasmic reticulum
SOCC	store-operated calcium channel
TRP	transient receptor potential cation channel
VGCC	voltage-gated calcium channel
VICC	voltage-independent calcium channel

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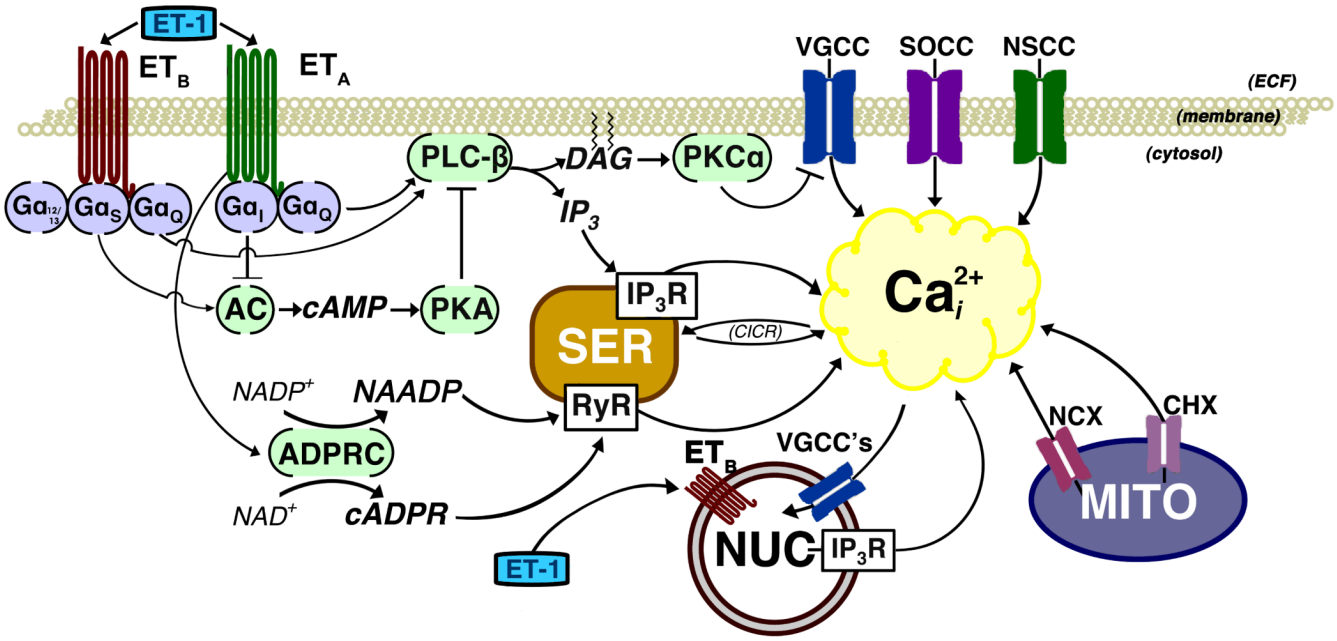

Figure 1.

This cartoon illustrates mechanisms that are linked to ET receptor-dependent increases in $[Ca^{2+}]_i$. In addition to the plasma membrane receptors, ET_B receptors in the nuclear membrane may mobilize calcium when activated by cytosolic ET-1. While the ET_A receptor is shown only in the plasma membrane, ET_B receptors are found in both the plasma and nuclear membranes. It is unknown if ET_A receptors are in the membrane of other organelles. Many of the same mechanisms are attributed to both ET_A- and ET_B-dependent increases in $[Ca^{2+}]_i$. Arrows represent activation; teed lines represent inhibition. Abbreviations: AC, adenylate cyclase; ADPRC, ADP-ribosyl cyclase; cADPR, cyclic ADP ribose; cAMP, cyclic adenosine monophosphate; CHX, calcium/hydrogen antiporter; CICR, calcium-induced calcium release; DAG, diacylglycerol; ECF, extracellular fluid; ET-1, endothelin-1; IP_3, inositol trisphosphate; IP_3R, inositol trisphosphate receptor; MITO, mitochondria; NAADP, nicotinic acid adenine dinucleotide phosphate; NAD^+, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; NCX, sodium/calcium exchanger; NSCC, non-selective cation channel; NUC, nucleus; PKA, protein kinase A; PKCα, protein kinase C-α; PLC-β, phospholipase C-β; RyR, ryanodine receptor; SER, smooth endoplasmic reticulum; SOCC, store-operated calcium channel; VGCC, voltage-gated calcium channel.

Table 1

Calcium channels, their characteristics, and pharmacological agents used to understand their function. Included are both voltage-dependent, voltage-independent, and endoplasmic reticular calcium channels. Abbreviations: V0.5, voltage of half-maximal activation; NSCC, non-selective cation channel; TRP, transient receptor potential channel; P2X, ATP-sensitive purinergic ion channel; 5-HT3, serotonin receptor subfamily 3.

Voltage-Gated Calcium Channels (VGCC's)					
Common Name	**Official Name**	**Characteristics**	**Specific Activators**	**Specific Inhibitors**	**Ref.**
L-type	$Ca_V1.2$	Cardiac and smooth muscle Ca^{2+} Channel. Regulates contraction. Moderate activation threshold ($V_{0.5}$= −10 mV). Relatively slow inactivation rate.	BAYK-8644	nifedipine, verapamil	[25]
N-type	$Ca_V2.2$	Neuronal Ca^{2+} Channel. Regulates neurotransmitter release. High activation threshold ($V_{0.5}$= +10 mV). Moderate inactivation rate (100-800 msec).	--	ω-conotoxin CVIA, ω-grammatoxin SIA	[25]
P/Q-type	$Ca_V2.1$	Neruonal Ca^{2+} Channel. Regulates neurotransmitter release. Moderate activation threshold ($V_{0.5}$= −10 mV). Inactivation rate varies by β subunit (0.09-1000 msec).	--	ω-conotoxin MVIIC, ω-agatoxin IIIA	[25]
R-type	$Ca_V2.3$	Neuronal Ca^{2+} Channel. Regulates Ca^{2+}-dependent gene expression and enzyme activity. High activation threshold ($V_{0.5}$= +5 mV). Fast inactivation rate (2.1-2.4 msec).	--	SNX-482, ω-PnTx3-3	[25]
T-type	$Ca_V3.1$	Neuronal/dendritic Ca^{2+} Channel. Regulates action potentials and subthreshold potential oscillations. Low activation threshold ($V_{0.5}$= −45 mV). Moderate inactivation rate (20-50 msec).	--	kurtoxin, mibefradil	[25]

Voltage-Independent Calcium Channels (VICC's)					
Abbr.	**Full Name**	**Characteristics**	**Activators**	**Inhibitors**	**Ref.**
NSCC's	Non-selective cation channels	Ion channels that lack specificity for a specific cation. Examples: NSCC-1 and NSCC-2, most TRP channels.	maitotoxin	LOE-908	[19]
LGCC's	Ligand-gated calcium channels	Ion channels activated by binding of a ligand to the channel. Examples: P2×, 5-HT3, and NACh receptors.	Varies by type	Varies by type	[26-28]
SOCC's	Store-operated calcium channels	Ca^{2+} channel activated by depletion of sarcoplasmic Ca^{2+} stores. Examples: STIM1/Orai complexes and TRPC channels.	SR Ca^{2+} depletion	SKF-96365, 2-APB	[29]

Receptors Mediating Intracellular Calcium Release					
Abbr.	**Full Name**	**Characteristics**	**Activators**	**Inhibitors**	**Ref.**
IP_3R	Inositol 1,4,5-trisphosphate Receptor	Tetrameric receptor in the endoplasmic reticular membrane that functions as a low-conductance cation channel; activated by IP_3.	IP_3	Xesto-spongin C	[30]
RyR	Ryanadine Receptor	Tetrameric receptor in the endoplasmic reticular membrane that functions as a high-conductance cation channel; activated by increased intracellular calcium.	Caffeine	Iberiotoxin	[31]

Table 2

Examples of physiological processes mediated by ET receptors that are dependent upon calcium. (A): ET_A receptor-dependent responses. (B): and ET_B receptor-dependent responses. These examples highlight the diverse responses caused by ET-1, and dependent on Ca^{2+}. Not only do the responses differ between cell type and tissue type, but between receptor subtype as well. No correlation exists between ET receptor subtype and either extracellular calcium influx or intracellular calcium release.

A: Calcium-Dependent Physiological Responses Mediated by ET_A Receptors				
Tissue/Cell Type:	**Response(s):**	**Calcium Influx**	**$[Ca^{2+}]_i$ Release**	**Ref.**
Neutrophils	Activation and degranulation	✓		[47,48]
Cardiac myocytes	Inhibition of C-type Natriuretic Peptide (CNP) signaling	✓	✓	[49]
Human bronchus	Release of intracellular Ca^{2+} stores, causing bronchoconstriction		✓	[50]
Thin limb, loop of Henle	Unknown; thought to regulate sodium and water reabsorption	✓	✓	[51]
Aortic smooth muscle	Vasoconstriction	✓	✓	[52]
Venous smooth muscle	Wave-like Ca^{2+} currents, ultimately causing venoconstriction	✓	✓	[53]
Human optic nerve head	Ca^{2+}-dependent proliferation	✓	✓	[54]
Mouse osteoblasts	Induces bone formation	✓	✓	[55]
Rat carotid body	Hypoxia up-regulates ET_A receptors, which increases mitogenesis	✓		[56]
Olfactory mucosa non-neuronal cells	Unknown; both transient and sustained Ca^{2+} entry	✓	✓	[57]

B: Calcium-Dependent Physiological Responses Mediated by ET_B Receptors				
Tissue/Cell Type:	**Response(s):**	**Calcium Influx**	**$[Ca^{2+}]_i$ Release**	**Ref.**
Neutrophils	Chemotactic neutrophil migration		✓	[47]
Human bronchus	Ca^{2+} influx followed by Ca^{2+} stores release, causing bronchoconstriction	✓	✓	[50]
Collecting duct	Inhibition of water reabsorption and Na^+-K^+-ATPase activity	✓	✓	[58,59]
Guinea pig gall bladder	Constriction		✓	[60]
Human umbilical vein	Ca^{2+} influx, causing venoconstriction	✓		[61]
Endothelial cells	Increased NO production and vasodilatation	✓	✓	[62]
Olfactory mucosa sensory neurons	Unknown; both transient and sustained Ca^{2+} entry	✓	✓	[57]