Uncoupling of Calcium Mobilization and Entry Pathways in Endothelin-stimulated Pituitary Lactotrophs*

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In cells expressing Ca\(^{2+}\)-mobilizing receptors, Ins\(_{1,3,4,5,6}\)P\(_6\) induced Ca\(^{2+}\) release from intracellular stores is commonly associated with extracellular Ca\(^{2+}\) influx. Operation of these two Ca\(^{2+}\) signaling pathways mediates thyrotropin-releasing hormone (TRH) and angiotensin II (AII)-induced prolactin secretion from rat pituitary lactotrophs. After an initial hyperpolarization induced by Ca\(^{2+}\) mobilization from the endoplasmic reticulum (ER), these agonists generated an increase in the steady-state firing of action potentials, further facilitating extracellular Ca\(^{2+}\) influx and prolactin release. Like TRH and AII, endothelin-1 (ET-1) also induced a rapid release of Ca\(^{2+}\) from the ER and a concomitant spike prolactin secretion during the first 3–5 min of stimulation. However, unlike TRH and AII actions, Ca\(^{2+}\) mobilization was not coupled to Ca\(^{2+}\) influx during sustained ET-1 stimulation, as ET-1 induced a long-lasting abolition of action potential firing. This lead to a depletion of the ER Ca\(^{2+}\) pool, a prolonged decrease in [Ca\(^{2+}\)]\(_i\), and sustained inhibition of prolactin release. ET-1-induced inhibition and TRH/AII-induced stimulation of Ca\(^{2+}\) influx and hormone secretion were reduced in the presence of the L-type Ca\(^{2+}\) channel blocker, nifedipine. Basal [Ca\(^{2+}\)]\(_i\), and prolactin release were also reduced in the presence of nifedipine. Furthermore, TRH-induced Ca\(^{2+}\) influx and secretion were abolished by ET-1, as TRH was unable to reactivate Ca\(^{2+}\) influx and prolactin release in ET-1-stimulated cells. Depolarization of the cells during sustained inhibitory action of ET-1, however, increased [Ca\(^{2+}\)]\(_i\), and prolactin release. These results indicate that L-type Ca\(^{2+}\) channel represents a common Ca\(^{2+}\) influx pathway that controls basal [Ca\(^{2+}\)]\(_i\), and secretion and is regulated by TRH/AII and ET-1 in an opposite manner. Thus, the receptor-mediated uncoupling of Ca\(^{2+}\) entry from Ca\(^{2+}\) mobilization provides an effective control mechanism in terminating the stimulatory action of ET-1. Moreover, it makes electrically active lactotrophs quiescent and unresponsive to other calcium-mobilizing agonists.

Typically, in anterior pituitary cells operated by calcium-mobilizing agonists, release of Ca\(^{2+}\) from intracellular Ca\(^{2+}\) stores is associated with extracellular Ca\(^{2+}\) influx. The coordinate actions of these two pathways provide long-lasting Ca\(^{2+}\) signals and secretion during sustained agonist stimulation (1).

Endothelins (ETs)\(^1\) are common calcium-mobilizing agonists for secretory pituitary cells and operate through ETA receptors (2, 3) coupled to phospholipase C (4) but not phospholipase D-dependent signaling pathways (5). In lactotrophs, the ET-1-induced release of Ca\(^{2+}\) from intracellular pools is associated with a rapid and transient increase in prolactin secretion (6, 7) followed by a prolonged inhibition to below basal levels (8). This bidirectional effect of a calcium-mobilizing agonist on secretion is uncommon among cells expressing phospholipase C-coupled receptors and is unique for cells expressing ETA receptors. The mechanism underlying the paradoxical action of ETs on hormone secretion is still unknown. It is unlikely that a rapid desensitization of ETA receptors could explain the inhibition of secretion observed in lactotrophs. Therefore, as the sustained phase in hormone secretion is affected in ET-1-stimulated lactotrophs, we addressed the alternate hypothesis that Ca\(^{2+}\) influx is uncoupled from Ca\(^{2+}\) mobilization in ET-1-stimulated lactotrophs, leading to depletion of the ER calcium pool and inhibition of hormone secretion.

To test this hypothesis, rat pituitary lactotrophs were employed. These cells exhibit spontaneous, extracellular Ca\(^{2+}\)-dependent action potential (AP) activity (9), which is tightly coupled to basal prolactin secretion (10). In addition to ETA receptors, lactotrophs express thyrotropin-releasing hormone (TRH) receptors (11), the calcium-mobilizing actions of which have been well characterized (12). Angiotensin II (AII) also stimulates prolactin release, presumably through activation of AT\(_{1B}\) receptors (13). Two calcium entry pathways are proposed to operate in lactotrophs: voltage-gated (VGCC) and store-operated (SOCC) calcium channels (12). Our results indicate that Ca\(^{2+}\) influx through VGCC rather than through SOCC is essential for the sustained secretagogue actions of TRH and AII. In contrast to these agonists, ET-1 inhibits voltage-gated Ca\(^{2+}\) influx, leading to a depletion of the ER calcium pool, a decrease in [Ca\(^{2+}\)]\(_i\), and prolonged inhibition of prolactin release.

MATERIALS AND METHODS

Chemicals—GnRH, TRH, AII, and ET-1 were obtained from Peninsula Laboratories (Belmont, CA). Fura 2-AM was purchased from Molecular Probes (Eugene, OR). All other chemicals were purchased from Sigma.

Cell Culture and Hormone Secretion—Anterior pituitary glands from adult female Sprague-Dawley rats obtained from Charles River Inc. (Wilmington, MA) were dispersed into single cells by a trypsin/DNase treatment procedure (14). All experiments were performed in either mixed pituitary cell populations or purified lactotroph populations. Cell purification of dispersed pituitary cells was done by sedimentation on a ficoll gradient as described previously (15). For cell column perfusion, 2 × 10\(^5\) cells were incubated with preswollen cytodex-1 beads in 60-mm
**RESULTS**

In cell column perfusion experiments, continuous stimulation with 100 nM ET-1 induced a rapid and transient increase in prolactin release (Fig. 1A), the amplitude of which was determined by agonist concentration (data not shown). The spike phase was followed by a sustained inhibitory phase, during which prolactin secretion was significantly below that observed in controls (Fig. 1A). Activation of TRH and AII receptors also induced a rapid spike-like pattern of prolactin release; however, unlike the ET-1 response, the spike was followed by a sustained plateau phase, in which prolactin secretion remained elevated above basal levels (Fig. 1B). To determine if the pattern of [Ca\(^{2+}\)]\(_i\) signaling in response to ET-1, TRH, and AII mirrors that of secretion, we monitored changes in [Ca\(^{2+}\)]\(_i\), in single rat lactotrophs using the membrane-permeant Ca\(^{2+}\) indicator dye, fura 2-AM. Endothelin-1 induced a rapid rise in [Ca\(^{2+}\)]\(_i\), followed by a decrease to below basal levels (Fig. 1C). In contrast, in TRH- and AII-stimulated lactotrophs, the spike phase of the [Ca\(^{2+}\)]\(_i\) response was followed by sustained plateau phase (Fig. 1D).

During perfusion of dispersed pituitary cells at a flow rate of 0.6 ml/min with the collection of 1-min fractions, the stimulatory action of ET-1 lasted for 3–5 min (Fig. 1A). This is in contrast to the [Ca\(^{2+}\)]\(_i\) spike, which was elevated above basal levels for about 1–2 min. To better compare the hormone release response to ET-1 with that of changes in [Ca\(^{2+}\)]\(_i\), the effects of ET-1 were further examined using a rapid perfusion system, in which the flow rate was adjusted to 1.0 ml/min, and 3-s fractions were collected. The secretory profile was then compared with an averaged [Ca\(^{2+}\)]\(_i\) response from lactotrophs stimulated with 100 nM ET-1. Under these conditions, the hormone release profile mirrored that of the changes in [Ca\(^{2+}\)]\(_i\) (Fig. 2).

This further supports the view that the pattern of ET-1-induced changes in [Ca\(^{2+}\)]\(_i\) determines that of prolactin release. Another difference in TRH/AII-versus ET-1-stimulated cells was related to the pattern of recovery after the termination of stimuli. The removal of TRH and AII was followed by a return in the [Ca\(^{2+}\)]\(_i\) (Fig. 3A) and secretory responses (data not shown) to basal levels within 1–2 min. In contrast, at least 30 min was required for the recovery of basal [Ca\(^{2+}\)]\(_i\) (Fig. 3B) and prolactin secretion (Fig. 3C) following ET-1 treatment. These data indicate that TRH and AII operate in a manner typical for Ca\(^{2+}\)-mobilizing agonists, inducing biphasic effects, which consist of an early spike and sustained plateau responses. Additionally, the stimulatory actions are terminated immediately upon their removal. In contrast, ET-1 exerts bidirectional effects on [Ca\(^{2+}\)]\(_i\), and secretion. This includes an early stimulation followed by a sustained inhibition, the latter being present.
for a prolonged period upon removal of the agonist.

Typical bidirectional effects of ET-1 on 

$$[\text{Ca}^{2+}]_{i}$$

and secretion were regularly observed in single lactotrophs bathed in Ca $$^{2+}$$-containing medium (Figs. 1–3). In cells bathed in Ca $$^{2+}$$-deficient medium, ET-1 induced a monophasic increase in prolactin secretion and $$[\text{Ca}^{2+}]_{i}$$ (Fig. 4, A and B). Thyrotropin-releasing hormone also induced a monophasic increase in $$[\text{Ca}^{2+}]_{i}$$ (Fig. 4C). Readdition of extracellular Ca $$^{2+}$$ in TRH- but not ET-1-stimulated cells lead to an immediate increase in $$[\text{Ca}^{2+}]_{i}$$. Depletion of extracellular Ca $$^{2+}$$ consistently reduced $$[\text{Ca}^{2+}]_{i}$$ and prolactin secretion, indicating that Ca $$^{2+}$$ entry is coupled to basal $$[\text{Ca}^{2+}]_{i}$$ and prolactin secretion in lactotrophs (Fig. 4, A–C). Thus, it is likely that a common Ca $$^{2+}$$ influx pathway controls basal $$[\text{Ca}^{2+}]_{i}$$ and secretion and is facilitated by TRH/AII and inhibited by ET-1.

Previously, it has been suggested that VGCC and SOCC participate in TRH-induced Ca $$^{2+}$$ mobilization in lactotrophs (reviewed in Ref. 12). To test for the involvement of VGCC in mediating the inhibitory action of ET-1 on secretion, the interaction between the dihydropyridine Ca $$^{2+}$$ channel blockers, nifedipine and nimodipine, and ET-1 was examined. The inhibitory action of ET-1 on prolactin release was decreased, but not

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**Fig. 2.** Comparison of the profiles of $$[\text{Ca}^{2+}]_{i}$$ response and prolactin (PRL) release in pituitary lactotrophs. Calcium tracing represents the computer-derived means of data from 47 lactotrophs. Fractions for prolactin release were collected every 3 s at a flow rate of 1 ml/min.

**Fig. 3.** Patterns of $$[\text{Ca}^{2+}]_{i}$$ and secretory responses in TRH- and ET-1-stimulated lactotrophs. A and B, effects of addition and removal of TRH and ET-1 on $$[\text{Ca}^{2+}]_{i}$$, in single lactotrophs. In B, the rate of sampling was 60/min during the first 5 min, followed by 3/min. C, stimulation, inhibition, and recovery of secretion in cells perfused at a flow rate of 0.6 ml/min, with a fraction collection of 1 per min. Bar indicates duration of agonist application.

**Fig. 4.** Effects of extracellular Ca $$^{2+}$$ depletion on basal and agonist-induced Ca $$^{2+}$$ responses and prolactin release in perfused pituitary cells. A, comparison of the effects of ET-1 on prolactin release in cells perfused with Ca $$^{2+}$$-deficient and Ca $$^{2+}$$-containing media. B and C, typical profiles of $$[\text{Ca}^{2+}]_{i}$$, in cells stimulated with 5-min pulses of TRH or ET-1 after removal of extracellular Ca $$^{2+}$$ (−Ca $$^{2+}$$). At the end of experiments, cells were again perfused with Ca $$^{2+}$$-containing medium (+Ca $$^{2+}$$).
Bidirectional Actions of ET-1 on Lactotrophs

Figure 5. Effects of ET-1 on prolactin (PRL) release in nifedipine-treated perifused pituitary cells. Shaded bar indicates the duration of ET-1 pulse. The dotted line illustrates the difference in the levels of inhibition induced by nifedipine and ET-1. Nifedipine was continuously present from the moment of application.

Figure 6. Changes in membrane potential ($V_m$) in ET-1 and TRH-stimulated cells. A, ET-1 induced an immediate cessation of action potential firing. Such inhibition was present continuously for at least 30 min following removal of ET-1. B, TRH-induced transient inhibition of firing, which was consistently followed by an increase in the frequency of firing when compared with that before TRH application.

Completely abolished, in the presence of nifedipine (Fig. 5) and nimodipine (data not shown). As the magnitude of ET-1-induced inhibition of prolactin release was reduced in the presence of dihydropyridines, it suggests that these VGCC blockers and ET-1 act at the same point to inhibit secretion. Nifedipine also reduced basal prolactin release in a manner comparable with that observed in cells depleted of extracellular Ca$^{2+}$ (Figs. 4 and 5). The effects of nifedipine were concentration dependent (Fig. 5, A versus B). At a higher (100 nM) concentration of nifedipine, ET-1 was still able to inhibit prolactin release, but the level of inhibition was only 10–20% of that observed in control cells (Fig. 5B). In TRH-stimulated cells, the plateau phase of [Ca$^{2+}$], i, response was also reduced by 80–90% in the presence of 100 nM nifedipine. These observations are in accord with the hypothesis that L-type voltage-gated channels are common Ca$^{2+}$ influx channels involved in the control of [Ca$^{2+}$], i, and secretion in unstimulated and agonist-stimulated lactotrophs. Thus, when the Ca$^{2+}$ influx through these channels is inhibited by nifedipine, it cannot be further inhibited by ET-1.

To further test this hypothesis, membrane potential was measured in single free-running lactotrophs. Under the conditions employed in this study, lactotrophs frequently exhibited spontaneous electrical activity, which was inhibited by addition of nifedipine (not shown). Spontaneous electrical activity was abolished in ET-1 as well as in TRH-stimulated cells (Fig. 6, A and B). However, the pattern of this inhibition differed between the two agonists. Thyrotropin-releasing hormone induced a transient hyperpolarization and cessation of AP firing followed by an increase in the spiking frequency. In contrast, ET-1 induced a long-lasting hyperpolarization with the cessation of AP activity, which returned 15–30 min following its removal. The level of hyperpolarization of lactotrophs by ET-1 was about $-80 \text{ mV}$ (Fig. 6), which is close to the equilibrium potential for potassium under our experimental conditions.

This suggests that inhibition of spontaneous electrical activity is indirect due to activation of a potassium channel, which hyperpolarizes the cells. Depolarization of cells by high potassium (Fig. 7) or the addition of Bay K 8644, an L-type calcium channel agonist (data not shown), increased [Ca$^{2+}$], i, and prolactin secretion, further supporting that inhibition of voltage-gated Ca$^{2+}$ entry is indirect.

During the ET-1-induced inhibition of electrical activity, TRH was unable to induce further hyperpolarization or action potential activity. In contrast, addition of ET-1 to cells continuously stimulated with TRH induced an inhibition of sustained extracellular Ca$^{2+}$-dependent [Ca$^{2+}$], i, response and prolactin release (Fig. 8, A and B). This experiment demonstrates that activation of ET receptors is required for the inhibitory action on Ca$^{2+}$ influx. Furthermore, the low amplitude of the spike [Ca$^{2+}$], i, and prolactin responses to ET-1 in TRH-stimulated cells (Fig. 8, A and B) as well as to ionomycin (data not shown) indicates that the ER calcium pool is almost depleted. The amplitudes of TRH-induced [Ca$^{2+}$], i, responses also decreased in cells continuously exposed to 100 nM ET-1 in Ca$^{2+}$-deficient (Fig. 8D) as well as Ca$^{2+}$-containing medium (data not shown). The amplitudes of TRH- and ionomycin-induced Ca$^{2+}$ responses were progressively reduced in cells stimulated with ET-1, indicating that the intracellular Ca$^{2+}$ pool is depleted within 10–15 min of stimulation (data not shown). Accordingly, application of TRH 20 min after stimulation with ET-1 did not increase prolactin secretion (Fig. 8C).

Discussion

The coupling of Ca$^{2+}$ influx to a Ca$^{2+}$ mobilization pathway is commonly observed in non-excitable and excitable cells operated by Ca$^{2+}$-mobilizing receptors. This coupling is crucial to the regulation of intracellular Ca$^{2+}$ homeostasis and Ca$^{2+}$-controlled cellular functions during sustained agonist stimula-
induced increase in [Ca$^{2+}$]_i (oscillatory versus non-oscillatory) determines the pattern of Ca$^{2+}$ entry in these cells, the pattern of Ca$^{2+}$ release from intracellular stores (oscillatory versus non-oscillatory) determines the pattern of AP firing and associated voltage-gated Ca$^{2+}$ entry (1). A family of Ca$^{2+}$-controlled potassium channels plays a critical role in the synchronization of Ca$^{2+}$ mobilization with the Ca$^{2+}$ entry process during agonist stimulation (9). The excitability of these cells, however, does not exclude the operation of SOCC; in several excitable cells, including lactotrophs, capacitative calcium entry has also been identified (26).

Pituitary lactotrophs and GH cell lines express several Ca$^{2+}$-mobilizing receptors that operate in a manner typical for this class of receptors, i.e. Ca$^{2+}$ mobilization is coupled to Ca$^{2+}$ entry. Within them, TRH receptors are the best characterized. They are coupled to a phospholipase C pathway through a G$_{q}$/G$_{11}$ protein (11). Activation of this pathway leads to a non-oscillatory biphasic increase in [Ca$^{2+}$]_i and hormone secretion, the early phase being dependent on Ca$^{2+}$ mobilization and the sustained phase on Ca$^{2+}$ entry (27–29) (Figs. 3A and 4C). Both voltage-gated and capacitative Ca$^{2+}$ entry have been proposed to participate in TRH-induced Ca$^{2+}$ influx in these cells. The role of VGCC and the pattern of TRH-induced electrical activity is well established (30, 31), whereas the operation of SOCC requires more detailed investigations (12). The other agonist for these cells, AII, signals through AT$_{1B}$ receptors that are also coupled to a phospholipase C pathway (13). Our results indicate that this receptor operates in a manner comparable with that observed during TRH action. Furthermore, lactotrophs express V$_{1b}$ vasopressin and oxytocin receptors, the activation of which are also associated with a biphasic pattern of Ca$^{2+}$ signaling and prolactin secretion (32, 33).

The ET receptor is an additional member of calcium-mobilizing receptors expressed in lactotrophs as well as in other pituitary cell types (34). The coupling of this receptor to a phospholipase C pathway in pituitary cells (4) provides a transient increase in [Ca$^{2+}$]_i, and hormone release (6). In contrast to TRH and AII action, however, the early stimulatory action of ET-1 is substituted with a prolonged inhibition of prolactin release. This was initially observed by Samson et al. (35) and subsequently confirmed by several other groups (6, 36, 37). In accord with the binding data (4), it was suggested that inhibition was mediated by ET$_A$ receptors (2–4). In addition to the pituitary cells, inhibition of prolactin release by ET-1 was also observed in human decidual cells (38), which express ET$_A$ receptors as well (39). Our data demonstrate that uncoupling of Ca$^{2+}$ influx from Ca$^{2+}$ mobilization underlies the bidirectional action of ETs on prolactin release. Thus, ET-1 exhibits two opposite effects on Ca$^{2+}$ signaling, stimulation via Ca$^{2+}$ mobilization and inhibition of Ca$^{2+}$ influx pathways. The lack of effects of TRH and AII to promote Ca$^{2+}$ influx in ET-1-stimulated cells and the ability of ET-1 to inhibit Ca$^{2+}$ entry in TRH/AII-stimulated cells suggest that these receptors control the same Ca$^{2+}$ influx pathway but show the opposite mode of regulation. Furthermore, the same Ca$^{2+}$ influx channels control basal [Ca$^{2+}$]_i and prolactin release. Several lines of evidence presented here and earlier indicate that Ca$^{2+}$ influx through l-type calcium channels rather than SOCC is controlled by these three calcium-mobilizing receptors. (i) Basal prolactin release is inhibited by nifedipine and by depletion of extracellular Ca$^{2+}$. Spontaneous electrical activity in lactotrophs and GH cells is also inhibited by nifedipine and requires extracellular Ca$^{2+}$ (40). (ii) TRH-induced electrical activity, Ca$^{2+}$ influx, and prolactin secretion in these cells are nifedipine-sensitive (27, 29, 30). (iii) ET-1 induces a prolonged inhibition of electrical activity and AP-driven Ca$^{2+}$ influx through VGCC. The ability of the L-type Ca$^{2+}$ channels blocker, nifedipine and nimodipine, to diminish the inhibitory action of ET-1 on secretion also suggests that ET-1 acts on VGCC to reduce Ca$^{2+}$ entry. (iv) Capacitative Ca$^{2+}$ entry should be activated automatically by depletion of the ER calcium pool in cells expressing SOCC. As shown here, there is a progressive depletion of the ER calcium pool in ET-1- and TRH-stimulated cells, but this action is accompanied with a reverse action on Ca$^{2+}$ influx by these two receptors, inhibition by ET-1 and stimulation by TRH.

The results also indicate that inhibition of Ca$^{2+}$ entry through L-type Ca$^{2+}$ channels is indirect, possibly due to activation of potassium channels, leading to a hyperpolarization and abolition of pacemaker activity. Recently, the role of large-conductance K$^+$ channels in ET-stimulated lactotrophs has been suggested (41). These channels are also activated by TRH and are responsible for a rapid inhibition of electrical activity in lactotrophs (9). Since these channels are controlled by [Ca$^{2+}$]_i, however, the inhibition of AP firing is transient (42) and may explain the early phase in hyperpolarization of these cells. Thus, the sustained inhibition of electrical activity must be controlled by another yet unidentified potassium channel. Future experiments should be directed toward identification of this channel, as well as the mechanism of its activation by ET$_A$.
receptors, such as direct coupling with G proteins or indirect coupling through intracellular messenger pathways (43, 44).

In the presence of nifedipine, ET-1 is still able to induce a minor inhibition of prolactin release. Also, about 10% of basal extracellular Ca²⁺-dependent hormone secretion is controlled by Ca²⁺ influx through nifedipine-insensitive calcium channels. This influx could be mediated by other VGCC subtypes, of which the T-type is the best characterized in these cells (45, 46). It is also possible that the residual, nifedipine-insensitive calcium influx is related to the other family of channels, such as a tetrodotoxin-insensitive sodium channel identified recently in lactotrophs and GH₃-immortalized cells (47). Both T-type VGCC and the novel sodium channels are capable of providing the pacemaker activity and are functionally connected to the control of firing of APs. This may provide the explanation for the occurrence of a nifedipine-insensitive Ca²⁺ influx in unstimulated cells and its inhibition and facilitation by ET-1 and TRH, respectively.

Our results also suggest that both ET-1- and TRH-induced Ca²⁺ mobilization leads to depletion of the ER Ca²⁺ pool but that the rate of depletion does not immediately mirror the [Ca²⁺], profiles. In cells bathed in Ca²⁺-deficient medium, application of TRH or ionomycin during the early phase of decreased [Ca²⁺], resulted in an additional release of Ca²⁺. Also, although Ca²⁺ influx is coupled to Ca²⁺ mobilization in TRH-stimulated cells, this pathway does not protect the cells from a decrease in the ER calcium pool, as documented by the subsequent application of ET-1 and ionomycin, but is sufficient to keep the steady-state plateau phase in [Ca²⁺], response. This is consistent with a model in which the [Ca²⁺], in TRH- and ET-1-stimulated lactotrophs represents the balance between Ca²⁺ mobilization and Ca²⁺ influx. In ET-1-stimulated cells, Ca²⁺ mobilization is activated, and AP-driven Ca²⁺ influx is inhibited, leading to the bidirectional (spike and sustained inhibitory) change in [Ca²⁺], and a complete depletion in the intracellular Ca²⁺ pool. In TRH-stimulated cells, both pathways are activated, leading to the biphasic (spike and plateau) change in [Ca²⁺], but accompanied by a progressive decrease in the intracellular Ca²⁺ pool to a new steady-state level. However, additional experiments are needed to define the cellular Ca²⁺ homeostasis in lactotrophs.

In summary, our results indicate that the signaling through ETₐ receptors is essential not only for stimulatory but also for inhibitory actions of ETs on [Ca²⁺], and prolactin secretion. The latter function of these receptors is mediated by the un-coupling of Ca²⁺ mobilization and voltage-gated Ca²⁺ entry pathways. Such uncoupling leads to a unique physiological situation for a Ca²⁺-mobilizing agonist: unresponsive cells. The ER calcium pool is depleted due to Ca²⁺-mobilizing action of the ET-receptor complex, and, without a capacitative refilling, other calcium-mobilizing agonists are unable to initiate Ca²⁺ mobilization. Prolonged hyperpolarization of lactotrophs also protects these agonists to activate voltage-gated Ca²⁺ influx in these cells. Finally, basal pacemaker activity and hormone secretion are also inhibited. This makes lactotrophs unresponsive, leading to a sustained hypoprolactinemia. In this regard, a transient stimulatory action of ET-1 probably represents only a first step in the development of a quiescent status of these cells.

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