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Integration of cytoplasmic calcium and membrane potential oscillations maintains calcium signaling in pituitary gonadotrophs

(gonadotropin-releasing hormone/calcium oscillations/membrane potential oscillations)

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ABSTRACT **Pituitary gonadotrophs exhibit spontaneous low-amplitude fluctuations in cytoplasmic calcium concentration ($[Ca^{2+}]_i$) due to intermittent firing of nifedipine-sensitive action potentials. The hypothalamic neuropeptide, gonadotropin-releasing hormone, terminates such spontaneous $[Ca^{2+}]_i$ transients and plasma-membrane electrical activity and initiates high-amplitude $[Ca^{2+}]_i$ oscillations and concomitant oscillations in membrane potential (V_m). The onset of agonist-induced $[Ca^{2+}]_i$ oscillations is not dependent on V_m or extracellular Ca^{2+} but is associated with plasma-membrane hyperpolarization interrupted by regular waves of depolarization with firing of action potentials at the peak of each wave. The V_m and Ca^{2+} oscillations are interdependent during continued gonadotropin-releasing hormone action (>3–5 min), when sustained Ca^{2+} entry is necessary for the maintenance of $[Ca^{2+}]_i$ spiking. The initial and sustained agonist-induced Ca^{2+} transients and V_m oscillations are abolished by blockade of endoplasmic reticulum Ca^{2+}-ATPase, consistent with the role of Ca^{2+} re-uptake by internal stores in the oscillatory response during both phases. Such a pattern of synchronization of electrical activity and Ca^{2+} spiking in cells regulated by Ca^{2+}-mobilizing receptors shows that the operation of the cytoplasmic oscillator can be integrated with a plasma-membrane oscillator to provide a long-lasting signal during sustained agonist stimulation.**

In many agonist-stimulated cells, the increase in cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) that represents the intracellular signal responsible for the activation of cellular functions occurs in an oscillatory manner (1). Two distinct mechanisms have been proposed to explain such periodic changes in $[Ca^{2+}]_i$ in agonist-stimulated cells: these have been termed the (*i*) plasma-membrane and (*ii*) cytoplasmic oscillators (2). Operation of the plasma-membrane oscillator depends on the electrical properties of the cells and results in Ca^{2+} entry through voltage-sensitive calcium channels (VSCC) (3). For example, the spontaneous firing of action potentials (APs) in growth hormone-secreting pituitary cells (GH cell lines) correlates with the resting level of $[Ca^{2+}]_i$; increases in the frequency of APs are followed by increases in the amplitude and duration of oscillations in $[Ca^{2+}]_i$ (4). Spontaneous oscillatory $[Ca^{2+}]_i$ patterns are also seen in normal pituitary somatotrophs (5) and lactotrophs (6) and are responsible for the extracellular Ca^{2+}-dependence of basal growth hormone and prolactin release *in vitro* (7). The actions of several hypothalamic neurohormones, such as growth hormone-releasing hormone, corticotropin-releasing factor, somatostatin, and dopamine, include facilitation or inhibition of the basal-membrane oscillatory activity (5, 8–11).

On the other hand, many agonists stimulate phospholipase C via distinct G protein (G_q or G_i) after binding to their specific plasma-membrane receptors (12), leading to the hydrolysis of phosphatidylinositol bisphosphate and generation of inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol (13, 14). In nonexcitable cells, the rise in $InsP_3$ is coupled to mobilization of intracellular Ca^{2+} and is associated with Ca^{2+} entry through pathway(s) that are voltage-insensitive (15). Such Ca^{2+} influx through still-uncharacterized pathway(s) is required to replace the Ca^{2+} pumped out during the sustained operation of the cytoplasmic oscillator in these cells (2).

Rat pituitary gonadotrophs exhibit both low-amplitude spontaneous $[Ca^{2+}]_i$ transients that are dependent on extracellular Ca^{2+} (16) and high-amplitude agonist-induced $[Ca^{2+}]_i$ oscillations (16–19) that are initiated by activation of the $InsP_3$/diacylglycerol pathway (20, 21). The spontaneous $[Ca^{2+}]_i$ oscillations are associated with operation of the membrane oscillator, which is based on random firing of APs. Gonadotropin-releasing hormone (GnRH) terminates the spontaneous activity of the membrane oscillator and initiates concomitant oscillations in membrane potential (V_m) and $[Ca^{2+}]_i$. Our studies describe an interrelationship between V_m and $[Ca^{2+}]_i$ oscillations during the initial and sustained responses to GnRH.

EXPERIMENTAL PROCEDURES

Experiments were done on anterior pituitary cells from 2-week ovariectomized adult female Sprague–Dawley rats (200–250 g) obtained from Charles River Laboratories. Isolated anterior pituitary cells were prepared by trypsin digestion and physical dispersion (7). The purification of gonadotrophs from castrated animals was done by sedimentation on Ficoll gradients. This procedure has been shown by reverse plaque assay to provide highly purified gonadotrophs (85–90%) (22).

Before electrophysiological experiments the culture medium was replaced by a solution containing 125 mM NaCl, 4 mM KCl, 2.5 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, and 10 mM Hepes 10 (pH 7.35). V_m was measured under current-clamp conditions with the perforated-patch technique. Microelectrodes were filled with a high K^+ solution (75 mM K_2SO_4/10 mM KCl/1 mM $MgCl_2$/10 mM Hepes, pH 7.2). The pipet was backfilled with the high K^+ solution to which nystatin (100–150 μM) was added. V_m was measured under current clamp conditions using a List-7 patch-clamp amplifier. Permanent records of both controlled current and V_m were made on analogue magnetic tape. In some experiments the holding current differed from zero. All

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Abbreviations: GnRH, gonadotropin-releasing hormone; V_m, membrane potential; $[Ca^{2+}]_i$, cytoplasmic Ca^{2+} concentration; AP, action potential; VSCC, voltage-sensitive Ca^{2+} channels; TG, thapsigargin; $InsP_3$, inositol 1,4,5-trisphosphate.

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experiments were done on 2- to 3-day cultured cells at 22–24°C.

For single-cell Ca^{2+} measurements, purified gonadotrophs were plated on 25-mm cover slips coated with poly(L-lysine) and loaded with 2 μM indo-1 AM for 60 min at 37°C. The cover slips were mounted on the stage of an inverted Diaphot microscope attached to an intracellular Ca^{2+} analysis system (Nikon). In terms of Ca^{2+} responses to GnRH, identified gonadotrophs from castrated females were more sensitive and responded more uniformly than those from normal females (16).

RESULTS

Membrane and Cytoplasmic Oscillators. We had previously observed that rat pituitary gonadotrophs exhibit low-amplitude spontaneous and extracellular Ca^{2+}-dependent $[Ca^{2+}]_i$ fluctuations and GnRH-induced high-amplitude $[Ca^{2+}]_i$ oscillations (16). Such $[Ca^{2+}]_i$ profiles in unstimulated and agonist-stimulated cells are associated with the electrical activity of the cells. Fig. 1*A Left* shows that gonadotrophs exhibit spontaneous firing of AP at a frequency that varies from cell to cell. Such spontaneous firing of AP was abolished by addition of the Ca^{2+}-channel antagonist nifedipine or when cells were exposed to Ca^{2+}-deficient medium but was not abolished by tetrodotoxin. Similarly, spontaneous $[Ca^{2+}]_i$ oscillations were abolished with nifedipine (Fig. 1*A Right*) but were not affected by tetrodotoxin. The parallelism in sensitivity of basal $[Ca^{2+}]_i$ fluctuations and random AP firing to extracellular $[Ca^{2+}]$ and dihydropyridines indicates that the spontaneous Ca^{2+} transients depend on the electrical excitability of the gonadotrophs. AP frequency is much higher (15–100 min^{-1}) than the frequency of spontaneous Ca^{2+} spiking (2–10 min^{-1}), and the variations in the frequency of APs create such $[Ca^{2+}]_i$ oscillations.

At low (0.1 nM) doses, GnRH induced base-line Ca^{2+} spiking (Fig. 1*B Right*) and switched the spontaneous firing of APs into a pattern composed of immediate membrane hyperpolarization followed by V_m oscillations that were accompanied by AP firing at the peak of each wave (Fig. 1*B Left*). The frequency of V_m oscillations was similar to that of the agonist-induced $[Ca^{2+}]_i$ transients; the averaged frequency of Ca^{2+} spiking triggered by 0.1 nM GnRH was 9 ± 4 min^{-1} (n = 53), and the averaged frequency of V_m oscillations was 9 ± 3 min^{-1} (n = 14). Higher doses of GnRH (1–100 nM) induced Ca^{2+} responses composed of two phases, a large early $[Ca^{2+}]_i$ peak and a subsequent prolonged phase composed of either high-frequency (25 ± 2 min^{-1}), low-amplitude (226 ± 23 nM; n = 18) $[Ca^{2+}]_i$ spikes (Fig. 1*C Right*) or a smooth $[Ca^{2+}]_i$ profile without spiking (in ≈50% of the cells; data not shown). Such high doses of GnRH caused an immediate and sustained hyperpolarization of the membrane that corresponded temporally with the prolonged phase of elevated $[Ca^{2+}]_i$ and was followed by regular, high-frequency oscillations in V_m (Fig. 1*C Left*).

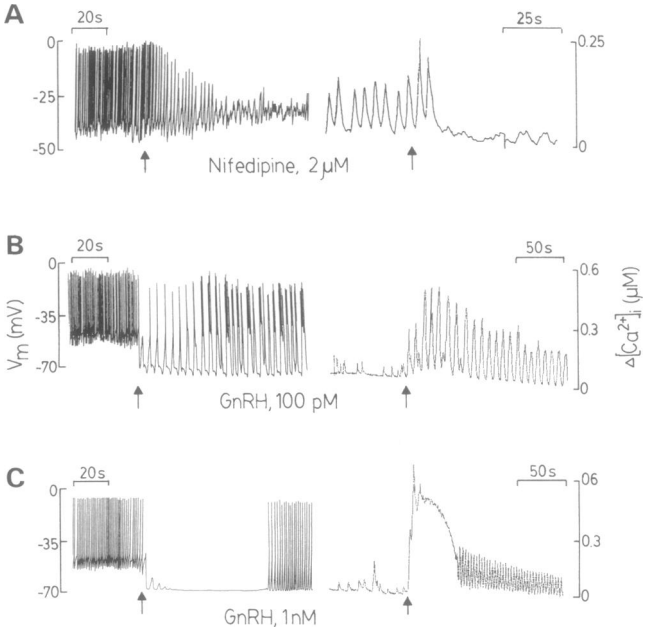

FIG. 1. V_m and $[Ca^{2+}]_i$ values in isolated gonadotrophs. (*A*) Effects of nifedipine on spontaneous APs (*Left*) and $[Ca^{2+}]_i$ oscillations (*Right*). (*B*) GnRH-induced V_m and $[Ca^{2+}]_i$ oscillations. (*C*) Biphasic V_m and $[Ca^{2+}]_i$ responses to a higher GnRH dose. Arrows indicate the time at which dose was applied.

Two Phases of Agonist-Induced Ca^{2+} Transients. The onset of regular, high-amplitude Ca^{2+} oscillations in GnRH-stimulated gonadotrophs is preceded by a number of progressively increasing $[Ca^{2+}]_i$ spikes before the maximal amplitude of the oscillatory response is reached (Fig. 1*B Right*). Thereafter, the $[Ca^{2+}]_i$ transients gradually decrease in amplitude toward the steady-state plateau oscillations in the presence of extracellular Ca^{2+}. In Ca^{2+}-deficient medium, however, Ca^{2+} transients triggered by low doses of GnRH are limited to 30–50 spikes over 3–5 min (Fig. 2*D*). The decrease in the amplitude of Ca^{2+} transients during prolonged stimulation by GnRH is associated with an increase in their frequency. This association is evident in an analysis of 12 experiments in which gonadotrophs were stimulated by 100 pM GnRH (Fig. 2 *A* and *B*). The high correlation between the frequency (expressed as the interspike interval) and amplitude of Ca^{2+} spiking (Fig. 2*C*) strongly suggests that these two phenomena are interrelated.

Additional experiments have also confirmed that such a pattern of Ca^{2+} spiking is functionally associated with emptying of an agonist-sensitive Ca^{2+} pool. Gonadotrophs were stimulated by GnRH (100 pM) with or without extracellular Ca^{2+} and then exposed to ionomycin (1 μM) at increased times after the initial stimulation (25, 75, 125, 175, 225, and 275 sec after GnRH application). Treatment with ionomycin can be used to estimate the residual capacity of the agonist-sensitive Ca^{2+} pool because ≈75% of the peak $[Ca^{2+}]_i$ response to a low concentration of the ionophore reflects release from the intracellular pool (23). The averaged amplitudes of such ionomycin-induced $[Ca^{2+}]_i$ responses in GnRH-prestimulated cells are shown in Fig. 2*E*; a progressive decrease in amplitude is observed in Ca^{2+}-deficient medium (200 nM, open bars) as well as in the presence of extracellular Ca^{2+} (1.25 mM, closed bars). On the other hand, participation of Ca^{2+} entry in ionomycin-induced $[Ca^{2+}]_i$ response (shown as the difference in amplitudes between open and closed bars, Fig. 2*E*) was not affected by prior GnRH treatment. Under both conditions, the amplitude of agonist-induced $[Ca^{2+}]_i$ spiking immediately before ionomycin treatment correlates with the amplitude of the Ca^{2+} response to this stimulus (Fig. 2*F*).

These data indicate that the pattern of agonist-induced Ca^{2+} spiking is determined by two factors, depletion of the agonist-sensitive Ca^{2+} pool and entry of Ca^{2+}. Although it does not fully replenish the agonist-sensitive Ca^{2+} pool during prolonged GnRH stimulation, the Ca^{2+} entry pathway is responsible for the sustained low-amplitude Ca^{2+} oscillations. Thus, the $[Ca^{2+}]_i$ response is composed of two temporal phases: the initial phase, which is triggered by release of Ca^{2+} and is relatively independent of extracellular Ca^{2+}, and the sustained phase, which depends highly on Ca^{2+} influx.

(In)dependence of Ca^{2+} Spiking from V_m. The striking similarity in the $[Ca^{2+}]_i$ and V_m oscillatory patterns and

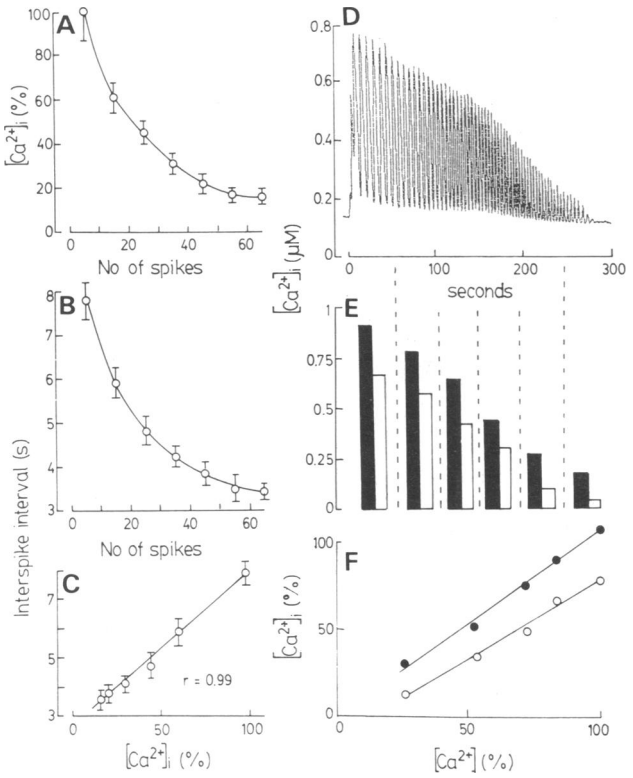

FIG. 2. Characteristics of agonist-induced $[Ca^{2+}]_i$ oscillations. (*A* and *B*) Decrease in amplitude and increase in frequency of Ca^{2+} spiking during prolonged GnRH (100 pM) stimulation. Data are means ± SE (n = 12) of the values normalized so that their maxima are expressed as 100, and the subsequent spikes are expressed as percentage of maximal amplitude (*A*); the corresponding frequency is expressed as the interspike interval (*B*). (*C*) Correlation between amplitude and frequency of Ca^{2+} spiking (r, Pearson's coefficient). (*D*) GnRH (100 pM)-induced $[Ca^{2+}]_i$ response in cells bathed in Ca^{2+}-deficient medium. (*E*) Averaged amplitudes (SE within 10%, n = 3) of ionomycin-induced $[Ca^{2+}]_i$ responses from GnRH-prestimulated cells. The initiation of Ca^{2+} spiking by GnRH is represented as 0, and the cells were exposed to ionomycin (1 μM) at increased times after initial stimulation (25, 75, 125, 175, 225, and 275 sec after GnRH application). (*F*) Correlation between amplitudes of agonist-induced $[Ca^{2+}]_i$ responses immediately before ionomycin treatment with amplitudes of ionomycin-induced $[Ca^{2+}]_i$ responses. Closed bars and circles, extracellular $[Ca^{2+}]$ = 1.25 mM; open bars and circles, extracellular $[Ca^{2+}]$ = 200 nM.

frequencies indicates that V_m oscillations depend on the rise in $[Ca^{2+}]_i$ in gonadotrophs, as in several other nonexcitable cell types operated by Ca^{2+}-mobilizing receptors (24, 25). Additional experiments have confirmed that V_m and $[Ca^{2+}]_i$ oscillations in gonadotrophs are synchronized due to the activity of a Ca^{2+}- and apamin-sensitive K^+ current (32, 33).

We used the Ca^{2+}-activated current to indirectly measure $[Ca^{2+}]_i$ under voltage-clamp conditions and to test the opposite hypothesis—i.e., whether the initial and sustained GnRH-induced $[Ca^{2+}]_i$ transients depend on V_m. At V_m values in the range of −100 to 0 mV, cells that were not stimulated by GnRH did not exhibit an oscillatory current. However, addition of GnRH activated an oscillatory current, with a frequency corresponding to that seen in both V_m and $[Ca^{2+}]_i$ oscillations at the same GnRH concentration. Two examples of such oscillatory currents are shown in Fig. 3*A*. In addition, in cells exposed to the pore-forming antibiotic nystatin, spontaneous V_m oscillations are abolished (Fig. 3*B*), and the resulting depolarization increases $[Ca^{2+}]_i$ (Fig. 3*C* left). In such "voltage-clamped" cells, GnRH stimulated Ca^{2+} transients of similar amplitude and frequency as in controls (Fig. 3*C Right*). These results indicate that although

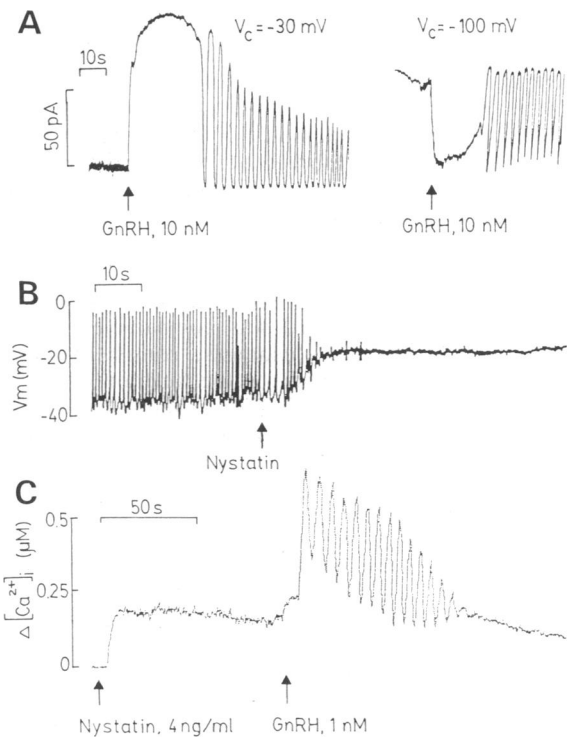

FIG. 3. Agonist-induced and nystatin-modulated oscillations in membrane current and $[Ca^{2+}]_i$. (*A*) GnRH-evoked oscillatory whole-cell membrane current in gonadotrophs, with reverse directions at holding potentials (V_c) of −30 and −100 mV. (*B*) Effect of nystatin on spontaneous APs. (*C*) $[Ca^{2+}]_i$ response of a single gonadotroph to nystatin and GnRH.

GnRH-induced $[Ca^{2+}]_i$ oscillations are associated with a complex pattern of electrical activity in gonadotrophs, the initiation of Ca^{2+} spiking is independent of V_m and, thus, does not depend on Ca^{2+} entry through voltage-sensitive pathways.

On the other hand, maintenance of the $[Ca^{2+}]_i$ oscillations is highly dependent on extracellular Ca^{2+} and V_m. Fig. 4*A* shows that the plateau-phase oscillatory $[Ca^{2+}]_i$ response elicited by high GnRH doses is abolished after EGTA appli-

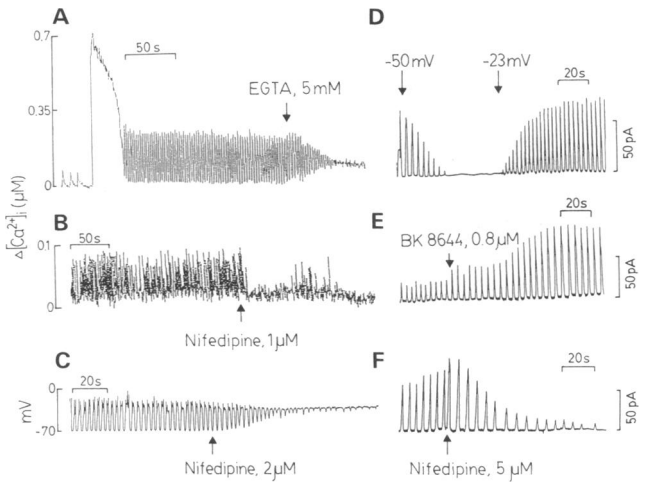

FIG. 4. Effects of extracellular Ca^{2+}, V_m, and dihydropyridines on sustained $[Ca^{2+}]_i$ oscillations induced by GnRH. (*A*) Abolition of the plateau oscillatory $[Ca^{2+}]_i$ response by EGTA. (*B* and *C*) Nifedipine-sensitivity of the sustained $[Ca^{2+}]_i$ and V_m oscillations. (*D*) Sensitivity of the oscillatory current to V_m. (*E* and *F*) Effects of dihydropyridines on the oscillatory current.

cation. The GnRH-induced current oscillations were also abolished within 5 to 15 min under voltage-clamp conditions at potentials negative to −25 mV. In the same cells, depolarization of the membrane to potentials positive to −25 mV caused recovery of the amplitude of the oscillatory current. The slow time course of this recovery phenomenon suggests that a mechanism involving modulation of the amplitude of $[Ca^{2+}]_i$ oscillations, rather than a voltage-sensitive activation of the Ca^{2+}-gated current, is responsible for the depolarization-dependent increase of current.

The range of potentials at which these voltage-sensitive changes in current occurred was comparable to that of activation of L-type Ca^{2+} channels, consistent with the involvement of these channels in the function of the cytoplasmic oscillator. Nifedipine (Fig. 4) and cobalt (data not shown), blockers of VSCC, abolished both $[Ca^{2+}]_i$ (Fig. 4*B*) and V_m (Fig. 4*C*) oscillations in gonadotrophs stimulated by GnRH. Complementary experiments under voltage-clamp conditions at an "intermediate" potential (−30 mV) revealed that nifedipine (Fig. 4*F*) and cobalt (data not shown) promptly inhibited the oscillatory current. Conversely, the dihydropyridine Ca^{2+} channel agonist Bay K 8644 increased the current amplitude (Fig. 4*E*). These data agree with the idea that VSCC participate in the sustained, extracellular Ca^{2+}- and V_m-dependent $[Ca^{2+}]_i$ oscillations. This Ca^{2+}-influx pathway plays an important part in maintaining the oscillatory response by ensuring Ca^{2+} availability for prolonged cycling. The record shown in Fig. 5 is from a cell that continued to oscillate for 40 min. In other cells stimulated by 1 nM GnRH, V_m oscillations continued for 2–3 hr.

Role of Endoplasmic Reticulum Ca^{2+}-ATPase in $[Ca^{2+}]_i$ Oscillations. Thapsigargin (TG), a blocker of the endoplasmic reticulum Ca^{2+}-ATPase, was used to examine the possible participation of this Ca^{2+} pump during the initial and sustained GnRH-evoked $[Ca^{2+}]_i$ oscillations. We had reported (16) that pretreatment of gonadotrophs with TG changes the oscillatory Ca^{2+} response to GnRH into a biphasic response. In accord with this observation, TG application immediately after initiation of $[Ca^{2+}]_i$ spiking by low doses of GnRH abolished the oscillatory response (Fig. 6*B*). In current-clamped cells, addition of TG also abolished V_m oscillations

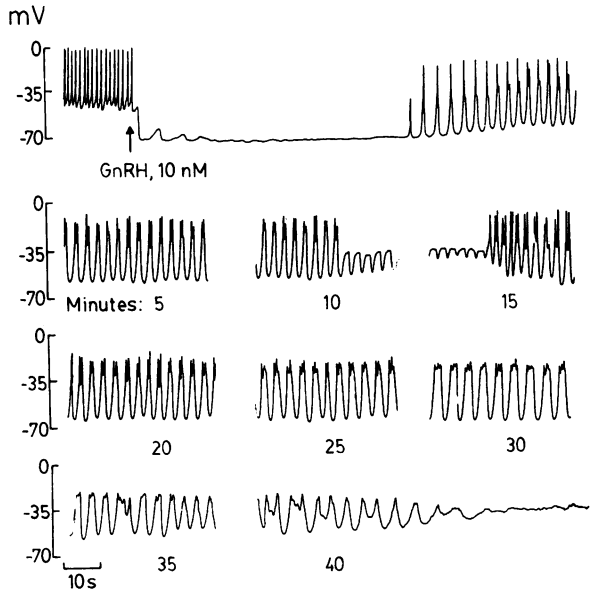

FIG. 5. V_m records from single gonadotrophs during continuous agonist stimulation. Cells were stimulated by 10 nM GnRH with normal extracellular $[Ca^{2+}]$ at room temperature. Eight segments are shown from the times indicated during continuous recording of V_m for 40 min.

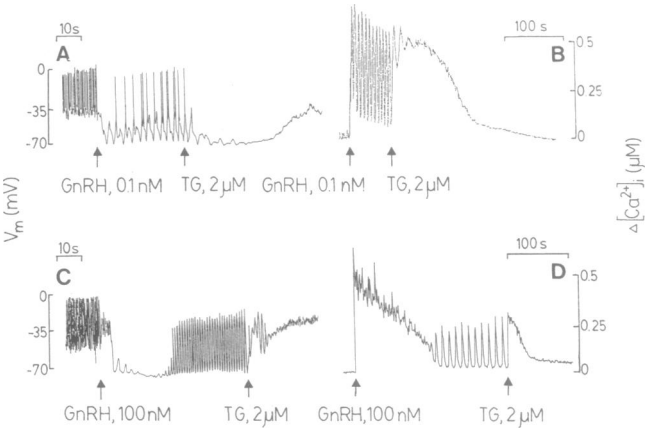

FIG. 6. Effects of the endoplasmic reticulum Ca^{2+}-ATPase blocker, TG, on GnRH-evoked V_m and $[Ca^{2+}]_i$ oscillations. (*A* and *B*) Effects of TG on V_m and $[Ca^{2+}]_i$ oscillations initiated by low GnRH doses. (*C* and *D*) TG sensitivity of the sustained V_m and $[Ca^{2+}]_i$ oscillations elicited by high GnRH doses.

(Fig. 6*A*), confirming the dependence of V_m changes on agonist-induced oscillatory release of Ca^{2+}. Similarly, the sustained extracellular Ca^{2+}-dependent $[Ca^{2+}]_i$ and V_m oscillations were abolished after TG application (Fig. 6 *C* and *D*).

Such an action of TG supports the model of a cytoplasmic oscillator in which intracellular pools discharge Ca^{2+} into the cytoplasm and subsequently reaccumulate Ca^{2+} from that source. These data also indicate that the sustained $[Ca^{2+}]_i$ oscillations are both initiated and driven by the same mechanism, although the source of Ca^{2+} has changed to the extracellular space. It is probable that Ca^{2+} entering the cytoplasm from the extracellular fluid is taken up by and released from the endoplasmic reticulum in an equilibrium-dependent manner, generating long-lasting oscillations with relatively constant frequency and amplitude that continue during agonist stimulation.

DISCUSSION

These data confirm that both plasma-membrane and cytoplasmic Ca^{2+} oscillators are operative in rat pituitary gonadotrophs; the former is spontaneously active and depends on Ca^{2+} entry through VSCC, and the latter are initiated by agonist-stimulated Ca^{2+} release that is associated with V_m oscillations. Thus, gonadotrophs do not differ from other pituitary cells, such as somatotrophs (5), lactotrophs (6), or GH cells (4), in which electrical excitability is associated with spontaneous oscillations in $[Ca^{2+}]_i$. In addition, initiation of the $[Ca^{2+}]_i$ response in gonadotrophs is similar to that seen in other cells operated by Ca^{2+}-mobilizing receptors, either excitable or nonexcitable. A common feature of cells operated by Ca^{2+}-mobilizing receptors, the association of changes in V_m with agonist-induced $[Ca^{2+}]_i$ responses (24, 25), is also present in gonadotrophs. The onset of the Ca^{2+} response is resistant to voltage-clamping and extracellular Ca^{2+} depletion in cells operated by Ca^{2+}-mobilizing receptors, including the gonadotrophs, indicating that neither voltage-sensitive processes nor an influx of Ca^{2+} is involved in initiation of the response (15). On the other hand, the sustained $[Ca^{2+}]_i$ oscillations are highly dependent on Ca^{2+} entry in gonadotrophs as in excitable lactotrophs, and GH cells, as well as in nonexcitable acinar cells.

A basic difference between the gonadotrophs and other cells operated by Ca^{2+}-mobilizing receptors is related to the pattern of the Ca^{2+} response and its integration with V_m. For example, lactotrophs and GH cells lines are excitable cells in

which spontaneous operation of the membrane oscillator is coupled to basal hormone secretion and is under both negative (dopamine) and positive (thyrotropin-releasing hormone) control. However, activation of Ca^{2+}-mobilizing receptors by thyrotropin-releasing hormone and release of Ca^{2+} does not occur in an oscillatory manner, as in gonadotrophs. Instead, thyrotropin-releasing hormone causes nonoscillatory amplitude-modulated $[Ca^{2+}]_i$ transients due to Ca^{2+} mobilization, followed by a sustained extracellular Ca^{2+}-dependent and nifedipine-sensitive plateau, (ref. 4; unpublished work). The spike phase of the Ca^{2+} response correlates temporally with the hyperpolarization phase triggered by a Ca^{2+}-dependent K^+ current. On the other hand, the plateau phase coincides with the period of recovery of firing of APs, with an increase in their frequency (26). Such a pattern of synchronization of electrical and biochemical events is not unique to lactotrophs and the family of GH cells because vasopressin modulates spontaneous electrical activity in aortic cells similarly (27).

The present data show that GnRH triggers a more complex pattern of Ca^{2+} responses in excitable gonadotrophs, in many respects similar to those observed in nonexcitable cells operated by Ca^{2+} mobilizing receptors, such as exocrine acinar cells (15, 25, 28). Although the nonexcitable acinar cells also exhibit agonist-induced oscillations that are synchronized with V_m oscillations, their Ca^{2+} entry pathway, which maintains the activity of the cytoplasmic oscillator while the intracellular Ca^{2+} store is gradually depleted by extrusion of the released ion through plasma-membrane transporters, is independent of the V_m status of the cells. Acinar cells do not express VSCC through which Ca^{2+} can enter under unstimulated conditions or during the operation of the cytoplasmic oscillator, and the Ca^{2+} influx pathways are probably channels that communicate between the extracellular space and the $InsP_3$-sensitive stores, or from some $InsP_3$-insensitive store to that released by the second messenger (1). Putative regulators of the activity of these pathways include inositol phosphates, notably $InsP_3$ and Ins 1,3,4,5-tetrakisphosphate and the extent of depletion of the pool(s) (ref. 29, see also ref. 30).

The present data show that the pattern of V_m and $[Ca^{2+}]_i$ oscillations in gonadotrophs is intermediate between those of typical excitable and nonexcitable cells. Like lactotrophs, gonadotrophs are excitable, but unlike lactotrophs, the spontaneous operation of their membrane oscillator is not coupled to basal hormone (gonadotropin) secretion (7, 16). Instead, stimulation of exocytosis is only associated with GnRH-induced activation of the cytoplasmic oscillator, as seen in nonexcitable acinar cells (25). The oscillatory rise in $[Ca^{2+}]_i$ switches the spontaneous firing of APs into a complex pattern of V_m oscillations that are synchronized with Ca^{2+} transients in a manner similar to those in nonexcitable pancreatic cells, but with periodic firing of APs at the peaks of the depolarization phases and consequent entry of Ca^{2+} through VSCC, as in excitable pancreatic β-cells (31). In the later cell type, injection of $InsP_3$ mimics the glucose action in activation of the oscillatory electrical activity, confirming that in this cell type, like in gonadotrophs, mobilization of intracellular Ca^{2+} initiates $[Ca^{2+}]_i$ spiking. The present data show that this pattern of synchronization of agonist-activated Ca^{2+} transients and V_m oscillations in excitable cells has a minor role during the initial phase of agonist-stimulation and intracellular Ca^{2+} mobilization but is essential for the sustained operation of the cytoplasmic oscillator, as the agonist-sensitive Ca^{2+} pool becomes depleted. Such coordinated and interdependent actions of the agonist-induced biochemical and electrical events thus maintain the long-lasting Ca^{2+} signal during GnRH action.

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