L-type Ca$^{2+}$ Channels and K$^+$ Channels Specifically Modulate the Frequency and Amplitude of Spontaneous Ca$^{2+}$ Oscillations and Have Distinct Roles in Prolactin Release in GH$_3$ Cells* 

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GH$_3$ cells showed spontaneous rhythmic oscillations in intracellular calcium concentration ([Ca$^{2+}$]) and spontaneous prolactin release. The L-type Ca$^{2+}$ channel inhibitor nimodipine reduced the frequency of Ca$^{2+}$ oscillations at lower concentrations (100 μM–1 μM), whereas at higher concentrations (10 μM), it completely abolished them. Ca$^{2+}$ oscillations persisted following exposure to thapsigargin, indicating that inositol 1,4,5-trisphosphate-sensitive intracellular Ca$^{2+}$ stores were not required for spontaneous activity. The K$^+$ channel inhibitors Ba$^{2+}$, Cs$^+$, and tetraethylammonium (TEA) had distinct effects on different K$^+$ currents, as well as on Ca$^{2+}$ oscillations and prolactin release. Cs$^+$ inhibited the inward rectifier K$^+$ current (K$_{IR}$) and increased the frequency of Ca$^{2+}$ oscillations. TEA inhibited outward K$^+$ currents activated at voltages above −40 mV (grouped within the category of Ca$^{2+}$ and voltage-activated currents, K$_{Ca,V}$) and increased the amplitude of Ca$^{2+}$ oscillations. Ba$^{2+}$ inhibited both K$_{IR}$ and K$_{Ca,V}$ and increased both the amplitude and the frequency of Ca$^{2+}$ oscillations. Prolactin release was increased by Ba$^{2+}$ and Cs$^+$ but not by TEA. These results indicate that L-type Ca$^{2+}$ channels and K$_{IR}$ channels modulate the frequency of Ca$^{2+}$ oscillations and prolactin release, whereas TEA-sensitive K$_{Ca,V}$ channels modulate the amplitude of Ca$^{2+}$ oscillations without altering prolactin release. Differential regulation of these channels can produce frequency or amplitude modulation of calcium signaling that stimulates specific pituitary cell functions.

There is increasing evidence for distinct roles of different spatial and temporal patterns of intracellular free Ca$^{2+}$ concentration ([Ca$^{2+}$]) in the regulation of cellular processes (1). Many cell types exhibit oscillations of [Ca$^{2+}$], that may be differentially modulated to produce highly specific intracellular signals. For example, the frequency of Ca$^{2+}$ oscillations has been shown to regulate secretion, whereas the amplitude of Ca$^{2+}$ oscillations has been shown to regulate gene expression in different cell systems (1, 2). The multifunctional enzyme calmodulin kinase II has been shown to be capable of decoding different patterns of Ca$^{2+}$ signaling into different functional responses (3).

Endocrine cells have the intrinsic capacity for extensive spontaneous activity that is independent of stimulation by external factors. In pituitary cells, this activity is characterized by membrane potential oscillations, action potentials, and Ca$^{2+}$ oscillations (4–7). It is likely that this spontaneous, intrinsic signaling plays a role in basal hormone release by pituitary cells and other endocrine cells, although this role has yet to be clearly defined (8–10). In addition, the individual components of this intrinsic signaling may be targets of modulation through which diverse signals can induce specific changes in cellular activity and hormone release.

The rat pituitary growth hormone- and prolactin-secreting GH$_3$ cell line is a useful and well studied model system for the study of pituitary cell signaling. GH$_3$ cells express L-type Ca$^{2+}$ channels as well as inwardly rectifying and Ca$^{2+}$ - and voltage-activated K$^+$ channels. The biophysical and pharmacological properties of these channels have been extensively characterized in previous studies (11–19). GH$_3$ cells also show spontaneous activity that is generated by the coordinated action of ion channels, Ca$^{2+}$ influx, Ca$^{2+}$ release from intracellular stores, and other second messengers including IP$_3$ and cAMP. In these and other pituitary cells, the relative contributions of each of these cellular signaling components to overall cellular activity may vary from cell to cell and under stimulated versus unstimulated conditions (20–22). It is well established that increases in [Ca$^{2+}$], directly mediate hormone release in GH$_3$ cells and other endocrine cell types (10, 23, 24). Oscillatory patterns of Ca$^{2+}$ signaling provide the opportunity for a cell to respond to individual components of a Ca$^{2+}$ signal (e.g. baseline [Ca$^{2+}$]), oscillation frequency, oscillation duration, or oscillation amplitude). The functional response of the cell may be different if it depends on a “frequency-modulated” signal versus an “amplitude-modulated” signal (1). Individual ion channels and second messengers may play specific roles in generating specific patterns of spontaneous Ca$^{2+}$ signaling and in turn may generate different patterns of hormone release.

In this study, we have used the combination of patch clamp measurements, fluorescence imaging of intracellular Ca$^{2+}$ concentration, and a sensitive enzyme-linked immunosorbent assay (ELISA) for prolactin to study the ionic mechanisms controlling hormone release from GH$_3$ cells. We have investigated the role of specific patterns of spontaneous Ca$^{2+}$ signaling in

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§ The abbreviations used are: IP$_3$, inositol 1,4,5-trisphosphate; ELISA, enzyme-linked immunosorbent assay; TEA, tetraethylammonium.
prolactin release from GH3 cells by using Ca\textsuperscript{2+} and K\textsuperscript{+} channel antagonists to modulate the patterns of Ca\textsuperscript{2+} signaling.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—GH3 cells, obtained from American Type Culture Collection, Manassas, VA (ATCC 82.1), were maintained in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum, penicillin (0.05 IU/ml), and streptomycin (50 \( \mu \)g/ml) and incubated in a humid atmosphere of 5% CO\textsubscript{2}, 95% O\textsubscript{2} at 37 °C. Cells were harvested once a week by treatment with a phosphate-buffered saline containing EDTA (1 mm) and reseeded at 20% original density, either into 6-well plates for prolactin release assays, 35-mm diameter culture dishes for electrophysiological studies, or poly-l-lysine-coated coverslips for Ca\textsuperscript{2+}-imaging studies. The incubation medium was changed every 2–3 days.

**Electrophysiological Recordings**—Single cells were voltage-clamped, and voltage-activated K\textsuperscript{+} channel activity was recorded from whole GH3 cells using a List EPC-7 patch-clamp amplifier. For the recording of K\textsubscript{ir-V} channel activity, cells were superfused with a solution containing 140 mM NaCl, 2.8 mM KCl, 2 mM MgCl\textsubscript{2}, 1 mM CaCl\textsubscript{2}, 10 mM HEPES, 3 mM glucose, 5 \( \times \) 10\textsuperscript{−4} M tetrodotoxin (pH 7.2 with NaOH). The recording electrode contained 140 mM KCl, 10 mM EGTA, 1 mM MgCl\textsubscript{2}, 3 mM Mg-ATP, 10 mM HEPES (pH 7.2 with KOH) (all from Sigma). The solution in the recording electrode contained 140 mM KCl, 10 mM EGTA, 2 mM MgCl\textsubscript{2}, 10 mM HEPES, 3 mM Mg-ATP (pH 7.2 with KOH). Currents were evoked by hyperpolarizing from a −40 mV holding potential (duration 1.5 s, frequency 0.03 Hz). No leak subtraction was applied. Capacitance compensations were achieved using the patch-clamp amplifier. Residual artifacts and leakage currents were nulled using a P/4 subtraction.

Whole-cell K\textsubscript{ir} current recordings were performed using extracellular solutions containing 140 mM KCl, 4 mM MgCl\textsubscript{2}, 1 mM CaCl\textsubscript{2}, 10 mM HEPES, 7 mM glucose, 5 \( \times \) 10\textsuperscript{−7} M tetrodotoxin (pH 7.2 with KOH).

**Measurement of [Ca\textsuperscript{2+}]i**—[Ca\textsuperscript{2+}]i was measured using a fluorescence imaging system that has previously been described in detail (25). Briefly, 0.5-mm-thick poly-L-lysine-coated glass coverslips were loaded with fura2 by incubation in 5 \( \mu \)M fura2-AM for 40 min. Cells were then washed and maintained in normal medium for 30 min before experimentation. Coverslips were excited with a mercury lamp through 340- and 380-nm band-pass filters, and fluorescence at 510 nm was recorded through a 10\texttimes\ or 20\texttimes\ objective with a silicon intensified target camera to an optical memory disc recorder. Images were then digitized and subjected to background subtraction and shading correction, after which [Ca\textsuperscript{2+}]i was calculated on a pixel-by-pixel basis, as described previously, by a frame grabber and image analysis board (Data Translation). Data acquisition and analysis software were written by Dr. Michael Sanderson. Tracings in all figures are based upon fluorescence of a 4 \( \times \) 4 pixel area located within each cell body.

**ELISA**—A competitive ELISA has been developed for measuring prolactin secreted by GH3 cells. The assay utilizes an antibody (raised in rabbit) against rat prolactin. Both antiserum (prolactin5-9) and standards (prolactin BP-3) were kindly provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). For the collection of samples, GH3 cells were seeded into 6-well tissue culture plates 2 days before experiments (0.5–0.7 million cells/well). Release experiments were conducted at 37 °C in a humidified incubator with 5% CO\textsubscript{2}. Immediately before each experiment, cells were washed gently with media (Dulbecco's modified Eagle's medium with 20 mM HEPES and 0.1% bovine serum albumin, pH 7.4, with NaOH). After washing, aliquots of media (1 ml) were added to each well for 0.5- to 15-min time points such that release could be monitored before, during, and after exposure to drugs. The amount of prolactin (ng/ml/10\textsuperscript{6} cells) released in 0.5 h in the presence of drugs was expressed as a percentage of release from the same cells during 0.5 h under control conditions before exposure to drugs. After incubation with the cells, each media aliquot was centrifuged at 1800 rpm at 4 °C then stored at −20 °C or assayed directly by ELISA for prolactin. For the competitive prolactin ELISA, 96-well Nunc-Immu-Maxisorp Plates from Life Sciences, Denver, CO were used. Each well was coated with prolactin by incubation of 100 \( \mu \)l of 0.1 mM NaHCO\textsubscript{3}, pH 9.5, containing 1 ng of prolactin for 20–24 h at 4 °C. Before assay, prolactin-coated plates were washed with assay buffer containing 0.5 mM NaCl, 20 mM NaH\textsubscript{2}PO\textsubscript{4}, 0.05% Tween 20, 0.5% bovine serum albumin, pH to 7.4, then incubated with assay buffer for 30 min at room temperature to remove prolactin binding weakly to the plate. After further washing with assay buffer, undiluted samples (100 \( \mu \)l) or standards (0.02–40 ng) dissolved in 100 \( \mu \)l of media were added to the wells, followed by the addition of 50 \( \mu \)l of prolactin antibody at a dilution of 1:40,000. After 20–24 h at room temperature, bound antibody was detected using peroxidase-conjugated anti-rabbit antibody (Vector, Burlingame, CA) with tetramethylbenzidine (Life Technologies, Inc.) as substrate. The peroxidase reaction was terminated by 1 N H\textsubscript{2}SO\textsubscript{4}, and absorbance was measured by a microplate reader (Molecular Devices) at 450 nm. To determine the amount of prolactin present in the samples, a standard curve was generated. The peak of maximum absorbance (corresponding to no prolactin added) was plotted against known amounts of prolactin (0.02–40 ng). All samples were assayed in quadruplicates from three separate determinations.

**RESULTS**

The majority of GH3 cells (approximately 70%, n > 1500 cells in 50 experiments) showed spontaneous oscillations in [Ca\textsuperscript{2+}]i. The pattern of these oscillations in [Ca\textsuperscript{2+}]i, varied considerably from cell to cell. Ca\textsuperscript{2+} oscillations had a periodicity ranging from 3–30 s and a peak amplitude ranging from 40–300 nM in different cells. Some cells showed Ca\textsuperscript{2+} oscillations with a relatively consistent frequency, amplitude, and shape (e.g. Fig. 1, Cell #2), whereas other cells showed a more random pattern of oscillations (e.g. Fig. 1, Cell #28). Increasing the temperature of the bath solution from room temperature to 37 °C increased the frequency of spontaneous Ca\textsuperscript{2+} oscillations but did not result in any qualitative changes in spontaneous Ca\textsuperscript{2+} oscillations or in their response to the conditions described below (data not shown).

Ca\textsuperscript{2+} oscillations were completely abolished in all cells during perfusion with medium containing no added Ca\textsuperscript{2+}, and they were restored immediately upon replacement of Ca\textsuperscript{2+} (n = 120 cells in three experiments, data not shown). We have previously shown that GH3 cells show predominantly L-type Ca\textsuperscript{2+} currents that are inhibited by nimodipine (19). Nimodipine inhibited spontaneous Ca\textsuperscript{2+} oscillations in a concentration-dependent fashion (Fig. 1). Nimodipine (1 \( \mu \)M) reduced the frequency of Ca\textsuperscript{2+} oscillations in some cells and abolished them in other cells. Interestingly, although 1 \( \mu \)M nimodipine reduced the frequency of Ca\textsuperscript{2+} oscillations in some cells, in most of these cells it did not significantly affect their amplitude. Nimodipine (10 \( \mu \)M) abolished Ca\textsuperscript{2+} oscillations in all cells (n = 100 cells in three experiments). Nimodipine (10 \( \mu \)M) also reduced spontaneous prolactin release by 32.9 ± 9.1% (see Fig. 9). The persistence of prolactin release despite the abolition of Ca\textsuperscript{2+} oscillations shows that a significant portion of the spontaneous hormone release does not require spontaneous Ca\textsuperscript{2+} signaling.

Exposure to 1 \( \mu \)M thapsigargin resulted in an increase in base-line [Ca\textsuperscript{2+}]i, and a slight increase in the frequency of Ca\textsuperscript{2+} oscillations in most cells (n = 120 cells in four experiments). Base-line [Ca\textsuperscript{2+}]i, returned to previous levels after 3–5 min in thapsigargin. After exposure to thapsigargin, Ca\textsuperscript{2+} oscillations continued to occur in 69% of cells showing spontaneous oscillations (59/87 cells in three experiments), although in some cells their amplitude and frequency were reduced (Fig. 2). The phospholipase C inhibitor U73122 had no significant effect on Ca\textsuperscript{2+} oscillations, suggesting that the active formation of IP3 is not required for spontaneous Ca\textsuperscript{2+} oscillations (n = 100 cells in three experiments, data not shown). The activity of both thapsigargin and U73122 were verified by the observation that each agent completely inhibited the rapid peak increase in [Ca\textsuperscript{2+}]i induced by thyrotropin-releasing hormone (data not shown). These results show that the activity of phospholipase C and the release of thapsigargin-sensitive intracellular Ca\textsuperscript{2+} are not required for spontaneous Ca\textsuperscript{2+} oscillations in most cells but may contribute to modulation of their frequency and amplitude.
The effect of nimodipine suggests that Ca\textsuperscript{2+} oscillations are generated by spontaneous depolarizations with resultant influx of Ca\textsuperscript{2+} through voltage-gated channels. Consistent with this hypothesis, spontaneous Ca\textsuperscript{2+} oscillations were highly sensitive to depolarization induced by increasing extracellular [K\textsuperscript{+}]. Increasing extracellular [K\textsuperscript{+}] by as little as 3 mM resulted in an increase in base-line [Ca\textsuperscript{2+}] as well as an increase in the frequency of Ca\textsuperscript{2+} oscillations (n = 100 cells in three experiments, not shown).

GH\textsubscript{3} cells display multiple types of K\textsuperscript{+} currents, including an inward rectifying (K\textsubscript{IR}) current and outward currents mediated by Ca\textsuperscript{2+} and voltage-activated channels (15–17, 26). For the purposes of this study, the different outward K\textsuperscript{+} currents recorded were grouped into a single category of Ca\textsuperscript{2+} and voltage-activated (K\textsubscript{Ca,V}) currents. KCa,V channel activity was recorded using the whole-cell patch-clamp configuration by depolarizing GH\textsubscript{3} cells held at -80 mV to between -50 and 60 mV in 10-mV increments (Fig. 3). Using the specified internal and external solutions (see “Experimental Procedures”), outward K\textsuperscript{+} currents were observed with a threshold of activation of approximately -40 mV. Different recording solutions (see “Experimental Procedures”) and hyperpolarizing steps from a -40 mV holding potential to between -50 and -120 mV (10 mV decrements) were used to specifically activate currents mediated by K\textsubscript{IR} channels. K\textsubscript{IR} channel activity recorded at more depolarized potentials than -40 mV was overwhelmed by currents through K\textsubscript{Ca,V} channels. K\textsubscript{IR} and K\textsubscript{Ca,V} currents were inhibited selectively by different agents (Fig. 4). TEA (1 mM) inhibited outward K\textsubscript{Ca,V} currents but not K\textsubscript{IR} currents. The outward K\textsuperscript{+} currents had both transient and sustained components; the inhibition of outward K\textsuperscript{+} current by TEA was most marked at the end of the voltage step, indicating a relatively selective block of the sustained current component. Bu\textsuperscript{2+} (1 mM) inhibited both K\textsubscript{IR} and K\textsubscript{Ca,V} (with no obvious specificity for the transient or sustained components), whereas Cs\textsuperscript{+} inhibited only K\textsubscript{IR} currents (Fig. 4).

Ca\textsuperscript{2+}, Bu\textsuperscript{2+}, and TEA also had distinct effects on Ca\textsuperscript{2+} oscillations. TEA (1 mM) induced a marked increase in the ampli-
KIR currents were activated by hyperpolarizing pulses (duration 1.5 s) to voltages between...

...increase in prolactin release. Our results show a significant effect on the amplitude of Ca$^{2+}$ oscillations as well as a slight increase in base-line [Ca$^{2+}$].

A complete list of steps involved in the process of KIR channel activation is provided...

...to voltages between −50 to 60 mV (10 mV increments) for 100 ms. Traces represent averaged currents recorded from 6 cells. B, relationship between the peak K$^{+}$ current amplitude and test pulse are shown graphically. Data were obtained from the same cells as in A. Vertical error bars, when bigger than symbols, represent ±S.E. C, K$_{ir}$ currents were activated by hyperpolarizing pulses (duration 1.5 s) to voltages between −50 to −120 mV (10 mV decrements) from a −40 mV holding potential. Equimolar RCI (120 ms) solutions were used to record K$_{ir}$ channel activity (see “Experimental Procedures”). Tracings represent currents averaged from 5 cells. D, the graph shows the current-voltage relationship of K$_{ir}$ channel activity recorded from the same cells as in C. Both peak ($\blacksquare$) and sustained (□) current amplitude is illustrated. Vertical error bars represent ± S.E.

...a significant increase in the frequency of Ca$^{2+}$ oscillations in GH3 cells are completely dependent on influx of Ca$^{2+}$ and in the majority of cells, do not require release of intracellular Ca$^{2+}$ from thapsigargin-sensitive stores. The efficacy of thapsigargin in depleting Ca$^{2+}$ stores was verified by the complete inhibition of the peak increase in [Ca$^{2+}$]$_i$, in response to thyrotropin-releasing hormone, as reported previously by Nelson and Hinkle (31). The frequency and amplitude of Ca$^{2+}$ oscillations were diminished in some cells in response to thapsigargin, suggesting that intracellular Ca$^{2+}$ stores may play a modulatory rather than a primary role in Ca$^{2+}$ oscillations. This modulatory role may involve Ca$^{2+}$-induced Ca$^{2+}$ release through interaction of Ca$^{2+}$ with the IP$_{3}$ receptor (28, 30, 32). Thapsigargin did abolish spontaneous Ca$^{2+}$ oscillations in a significant proportion of cells (approximately 30%), suggesting that there is a subset of cells whose spontaneous Ca$^{2+}$ signaling does require intracellular Ca$^{2+}$ release from thapsigargin-sensitive stores. A similar dependence on IP$_{3}$-sensitive intracellular Ca$^{2+}$ stores in a subset of GH3 cells is suggested by Varney et al. (33), who found that chronic treatment with Li$^{2+}$, which reduces basal levels of IP$_{3}$, decreased the number of cells showing spontaneous Ca$^{2+}$ oscillations.

The effects of nimodipine indicate that spontaneous Ca$^{2+}$ oscillations are generated by influx of Ca$^{2+}$ through L-type channels that have previously been shown to be inhibited by nimodipine in GH3 cells (19). At lower concentrations, nimodipine often reduced the frequency of Ca$^{2+}$ oscillations without significantly altering their amplitude. This observation suggests that the frequency of Ca$^{2+}$ oscillations is dependent upon the activation state of Ca$^{2+}$ channels, whereas the base-to-peak amplitude of spontaneous Ca$^{2+}$ oscillations is an all-or-none phenomenon that is not regulated by the state of Ca$^{2+}$ channels under unstimulated conditions. The central role of Ca$^{2+}$ channels in the overall activity of the cell makes them a logical target for inhibitory or excitatory modulation by external ligands. The observations that somatostatin receptors and expressed opioid receptors in GH3 cells modulate Ca$^{2+}$ channels is consistent with their role as a target for receptor-mediated modulation (19, 34).

Nimodipine inhibited basal prolactin release by approximately 30%, indicating that a significant proportion of basal prolactin release is stimulated by spontaneous Ca$^{2+}$ signaling. However, the persistence of prolactin release in the absence of any detectable Ca$^{2+}$ signaling shows that a high proportion of basal prolactin release is independent of spontaneous Ca$^{2+}$ oscillations. This finding is consistent with the report of Masumoto et al. (10), who found that basal exocytosis continued...
in the absence of increases in \([\text{Ca}^{2+}]_i\), in pituitary gonadotrophs. It is likely that this represents an unregulated pathway of secretion that has been reported in GH3 cells (35) and other cell types.

Different K⁺ channel antagonists have distinct effects on \([\text{Ca}^{2+}]_i\) signaling in GH3 cells. TEA evoked a dramatic increase in the amplitude of \([\text{Ca}^{2+}]_i\) oscillations; this effect is likely because of prolongation of the action potential and subsequent increase
in the action potential-induced influx of Ca$^{2+}$. This is consistent with the relatively selective effect TEA has on sustained outward K$^+$ currents recorded from GH3 cells, with little effect on the A current (15). Interestingly, the large increase in the amplitude of Ca$^{2+}$ oscillations was not associated with a large increase in base-line [Ca$^{2+}$]$_i$, showing that base-line [Ca$^{2+}$]$_i$ is regulated by a mechanism other than TEA-sensitive outward K$^+$ currents. Both Ba$^{2+}$ and Cs$^+$ increased the frequency of Ca$^{2+}$ oscillations, whereas TEA did not. These results indicate that the inward rectifier K$^+$ channel plays a primary role in setting the frequency of spontaneous Ca$^{2+}$ oscillations. They are consistent with the results of Barros et al. (36, 37), who report a primary role for the inward rectifier in the regulation of GH3 cell excitability. Base-line [Ca$^{2+}$], was increased dramatically by Ba$^{2+}$, and to a lesser extent, by Cs$^+$ and TEA. These results suggest that Ba$^{2+}$ modulates base-line [Ca$^{2+}$], by a mechanism that is distinct from the inward rectifier, and as discussed above, distinct from TEA-sensitive outward K$^+$ currents.

Prolactin release was also differentially modulated by different K$^+$ channel antagonists. Despite the fact that TEA caused a marked increase in the amplitude of Ca$^{2+}$ oscillations and a significant increase in average [Ca$^{2+}$]$_i$, it did not increase prolactin release. By contrast, Cs$^+$, which increased the frequency of Ca$^{2+}$ oscillations but caused a much smaller increase in average [Ca$^{2+}$]$_i$ than TEA, did cause a significant increase in prolactin release. These findings are consistent with the stimulation of hormone release by an increase in the frequency of spontaneous Ca$^{2+}$ oscillations but not in their amplitude. The marked effect of Ba$^{2+}$ suggests that the combination of increased base-line [Ca$^{2+}$]$_i$ and increased Ca$^{2+}$ oscillation frequency can cause a much greater increase in hormone release than increased Ca$^{2+}$ oscillation frequency alone.

Simultaneous measurements of membrane capacitance and [Ca$^{2+}$]$_i$, by multiple investigators have provided strong evidence that each oscillatory increase in [Ca$^{2+}$]$_i$, is capable of evoking secretion of hormone (24, 38–42). Our results suggest that increasing the peak [Ca$^{2+}$]$_i$ associated with each sponta-
neous Ca\(^{2+}\) oscillation does not increase hormone release; this may be because there is a maximum number of vesicles released per Ca\(^{2+}\) oscillation once the peak [Ca\(^{2+}\)] has reached a certain threshold or because buffering of Ca\(^{2+}\) prevents this increased peak [Ca\(^{2+}\)], from being sensed by the secretory apparatus. By contrast, the increase in hormone release associated with an increase in Ca\(^{2+}\) oscillation frequency suggests that modulation of Ca\(^{2+}\) oscillation frequency does represent a mechanism for stimulation of hormone release. Increasing basal-line [Ca\(^{2+}\)], in addition to increasing Ca\(^{2+}\) oscillation frequency, results in a further increase in prolactin release. Possible explanations for the specific effects of base-line [Ca\(^{2+}\)], and oscillation frequency on secretion include direct effects on the exocytotic trigger mechanism (43) or the recruitment of additional pools of vesicles with different Ca\(^{2+}\) sensitivities (44, 45).

\textit{Ca^{2+}} channels, K\(^{+}\) channels, Ca\(^{2+}\) influx, and intracellular Ca\(^{2+}\) release play specific roles in the generation of spontaneous activity in \(GH_3\) cells. In turn, specific characteristics of spontaneous Ca\(^{2+}\) signaling, namely base-line [Ca\(^{2+}\)], and the frequency of Ca\(^{2+}\) oscillations, are correlated with changes in hormone release. Our studies identify L-type \(Ca^{2+}\) channels and the inward rectifier K\(^{+}\) channel as key components of the cellular signaling machinery whose modulation results in the specific changes in the patterns of spontaneous cellular activity that regulate changes in basal prolactin release. TEA-sensitive outward K\(^{+}\) currents may affect other cellular processes through amplitude-modulated Ca\(^{2+}\) signaling.

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