Dependence of Pituitary Hormone Secretion on the Pattern of Spontaneous Voltage-gated Calcium Influx

CELL TYPE-SPECIFIC ACTION POTENTIAL SECRETION COUPLING

Received for publication, June 11, 2001, and in revised form, July 13, 2001
Published, JBC Papers in Press, July 16, 2001, DOI 10.1074/jbc.M105386200

Fredrick Van Goor‡, Dragoslava Zivadinovic, Antonio J. Martinez-Fuentes, and Stanko S. Stojilkovic§
From the Endocrinology and Reproduction Research Branch, NICHD, National Institutes of Health, Bethesda, Maryland 20892-4510

In excitable cells, voltage-gated calcium influx provides an effective mechanism for the activation of exocytosis. In this study, we demonstrate that although rat anterior pituitary lactotrophs, somatotrophs, and gonadotrophs exhibited spontaneous and extracellular calcium-dependent electrical activity, voltage-gated calcium influx triggered secretion only in lactotrophs and somatotrophs. The lack of action potential-driven secretion in gonadotrophs was not due to the proportion of spontaneously firing cells or spike frequency. Gonadotrophs exhibited calcium signals during prolonged depolarization comparable with signals observed in somatotrophs and lactotrophs. The secretory vesicles in all three cell types also had a similar sensitivity to voltage-gated calcium influx. However, the pattern of action potential calcium influx differed among three cell types. Spontaneous activity in gonadotrophs was characterized by high amplitude, sharp spikes that had a limited capacity to promote calcium influx, whereas lactotrophs and somatotrophs fired plateau-bursting action potentials that generated high amplitude calcium signals. Furthermore, a shift in the pattern of firing from sharp spikes to plateau-like spikes in gonadotrophs triggered luteinizing hormone secretion. These results indicate that the cell type-specific action potential secretion coupling in pituitary cells is determined by the capacity of their plasma membrane oscillator to generate threshold calcium signals.

Although anterior pituitary secretory cells are derived from the same progenitor cells, they differ with respect to their secretory patterns in vitro and in vivo. In vitro, basal prolactin (PRL) and growth hormone (GH) secretion from pituitary fragments, dispersed pituitary cells, and immortalized lacto-somatotrophs is high and is dependent on the extracellular calcium concentration (1–4). In contrast, basal luteinizing hormone (LH) secretion is low and not dependent on the extracellular calcium concentration (1). In vivo, animals bearing ectopic pituitary grafts release high levels of PRL and low levels of LH for a prolonged period, leading to pseudo-pregnancy (5). Because of the high levels of basal GH and PRL secretion, it is not surprising that lactotrophs and somatotrophs are under negative hypothalamic control by Gia-coupled dopamine and somatostatin receptors, in addition to positive control by Ca2+-mobilizing and Gc-coupled receptors, such as GH-releasing hormone and thyrotropin-releasing hormone receptors. On the other hand, LH secretion from gonadotrophs is under positive hypothalamic control by Ca2+-mobilizing receptors, including gonadotropin-releasing hormone (GnRH) and endothelin-A, but no inhibitory hypothalamic factor has been identified (6, 7).

It is not known what endows lactotrophs and somatotrophs, but not gonadotrophs, with the ability to secrete high levels of hormone in the absence of any stimuli. One possibility is that lactotrophs and somatotrophs fire spontaneous action potentials (APs) that are capable of driving sufficient Ca2+ entry to stimulate hormone secretion, whereas gonadotrophs are quiescent in the absence of any stimuli. Consistent with this, cultured somatotrophs (8, 9), lactotrophs (10, 11), and immortalized GH cells (12–16), as well as in situ somatotrophs (17), spontaneously fire APs, and the majority of unstimulated male rat gonadotrophs are quiescent (18). In ovariectomized rats, however, gonadotropin secretion remained low despite the observation that about 50% of the cells examined exhibited spontaneous AP firing (1, 19). These observations raise the possibility that the nature of spontaneous AP firing, such as Ca2+-dependent versus Na+-dependent spiking, or variations in the proportion of excitable cells, and/or frequency of spontaneous firing account for the cell type-specific patterns of basal hormone secretion. Finally, the differences in the patterns of basal hormone secretion may be due to differences in the ability of voltage-gated Ca2+ influx (VGCI) to increase intracellular calcium concentration ([Ca2+]i) and stimulate secretion in spontaneously active cells. In male gonadotrophs, for example, short membrane depolarization and the ensuing increase in [Ca2+]i do not stimulate exocytosis (20), whereas a prolonged membrane depolarization by high potassium is sufficient to stimulate secretion in several anterior pituitary cell types, including gonadotrophs (1, 21). Thus, the profile of the AP wave form, i.e. the AP duration, may determine the amplitude of the [Ca2+]i and secretory responses.

In the present study, we examined the patterns of AP-driven Ca2+ entry and their relationship to basal hormone secretion in each cell type under identical culture and recording conditions. Spontaneous electrical membrane activity and [Ca2+]i were recorded simultaneously to determine the ability of AP firing in each cell type to drive VGCI. To monitor basal hormone secre-
and its dependence on AP-driven Ca\(^{2+}\) entry at a similar time scale to that used in electrophysiological experiments, a rapid perfusion system was used. Our results indicate specific profiles of the AP wave forms in three cell types, and their ability to drive Ca\(^{2+}\) influx through voltage-gated Ca\(^{2+}\) channels (VGCCs) accounts for the cell type-specific patterns of basal hormone secretion. Specifically, gonadotrophs fired sharp, high amplitude APs with a limited capacity to drive Ca\(^{2+}\) influx, whereas lactotrophs and somatotrophs exhibited plateau-bursting activity that had a high capacity to drive Ca\(^{2+}\) entry.

**EXPERIMENTAL PROCEDURES**

**Cell Cultures and Treatments**—Experiments were performed on anterior pituitary cells from normal postpuberal female Harlan Sprague-Dawley rats obtained from Taconic Farm (Germantown, NY). Pituitary cells were dispersed as described previously (22) and cultured as mixed cells or enriched lactotrophs, somatotrophs, and gonadotrophs in medium 199 containing Earle’s salts, sodium bicarbonate, 10% heat-inactivated horse serum, and antibiotics. A two-stage Percoll discontinuous density gradient procedure (22) was used to obtain enriched lactotroph and somatotroph populations. Somatotrophs were further identified by their cell-specific morphology and by the addition of thyrotropin-releasing hormone. In enriched lactotroph populations, lactotrophs were further identified by their cell-type-specific morphology and by the addition of thyrotropin-releasing hormone. Gonadotrophs were initially identified by their cell type-specific morphology and, subsequent to experimentation by addition of GnRH, which stimulates small conductance, Ca\(^{2+}\)-activated K\(^{+}\) current and [Ca\(^{2+}\)]\(i\), oscillations only in gonadotrophs (23, 24).

Hormone secretion was monitored using rapid cell column perfusion experiments as previously described (25). Briefly, 1.5 \(\times 10^7\) cells were incubated with preswollen cytodex-1 beads in 60-mm Petri dishes for 2 days. The beads were then transferred to 0.5-ml chambers and perfused with Hanks’ M199 containing 20 mM HEPES and 0.1% bovine serum albumin for 2 h at a flow rate of 0.8 ml/min at 37 °C to establish a stable basal secretion. During the experiment, 1-min fractions were collected, stored at −20 °C, and later assayed for GH, PRL, and LH content using radioimmunoassay. All reagents and standards were provided by the National Pituitary Agency and Dr. Parlow. Standard curves for three radioimmunoassays were constructed in a concentration range of 1–100 ng/ml, and the displacement of labeled hormones with unlabeled hormones was done at 30% specific binding. The averaged IC\(_{50}\) was 6.30 ± 0.44 (n = 9), 6.52 ± 0.48 (n = 9), and 6.93 ± 0.73 (n = 8) ng/ml for GH, PRL, and LH, respectively, indicating similar sensitivity of three radioimmunoassays. To account for differences in the total number of hormone secreting cell types found in the anterior pituitary, hormone content from the same samples was measured and then normalized to the percentage of each cell type occurring in mixed cell populations.

**Immunocytochemistry of Rat Anterior Pituitary Cells**—To normalize hormone secretion to the total number of each cell type in the anterior pituitary, immunostaining of GH, LH, and PRL was performed using a avidin-biotin (ABC) peroxidase method. Dispersed cells were plated at a density of 200,000 cells/slide, fixed in Bouin’s fluid for 20 min, thoroughly washed, dehydrated, and kept dry at −70 °C. On the day of immunocytochemical processing, fixed cells were sequentially rehydrated, treated with 3% H\(_2\)O\(_2\), rinsed in phosphate-buffered saline, and then coated with Sylgard (Dow Corning Corporation, Midland, MI) to reduce pituitary capacitance. Pipette tips were briefly immersed in amphotericin B-free solution and then backfilled with the amphotericin B-containing solution. A series resistance of <15 MΩ was reached 10 min following the formation of a gigaohm seal (seal resistance > 5 GΩ) and remained stable for up to 1 h. Pulse generation, data acquisition, and analysis were done with a PC equipped with a Digidata 1200 A/D interface in conjunction with Clampex 8 (Axon Instruments).

For recording V\(_m\), the extracellular medium contained 120 mM NaCl, 2 mM CaCl\(_2\), 2 mM MgCl\(_2\), 4.7 mM KCl, 0.7 mM MgSO\(_4\), 10 mM glucose, and 10 mM HEPES (pH adjusted to 7.4 with NaOH), and the pipette solution contained 50 mM KCl, 90 mM K\(^+\) aspartate, 1 mM MgCl\(_2\), and 10 mM HEPES (pH adjusted to 7.2 with KOH). The bath contained <500 µl of saline and was continuously perfused at a rate of 2 ml/min using a gravity-driven perfusion system.

**Electrophysiological Measurements**—Current and voltage clamp recordings were performed at room temperature using an Axopatch 200 B patch clamp amplifier (Axon Instruments, Foster City, CA) and were low pass filtered at 2 kHz. Membrane potential (V\(_m\)) was measured using the perforated patch recording technique (26). Briefly, an amphotericin B (Sigma) stock solution (60 mg/ml) was prepared in Me\(_2\)SO and stored for up to 1 week at −20 °C. Just prior to use, the stock solution was diluted into pipette solution and sonicated for 30 s to yield a final amphotericin B concentration of 240 µg/ml. Patch electrodes were fabricated by pulling a borosilicate glass (outer diameter, 1.5 mm; World Precision Instruments, Sarasota, FL) using a Flaming Brown horizontal puller (P-87; Sutter Instruments, Novato, CA). Electrodes were heat polished to a final tip resistance of 3–6 MΩ and then coated with Silgard (Dow Corning Corporation, Midland, MI) to reduce pipette capacitance. Pipette tips were briefly immersed in amphotericin B-free solution and then backfilled with the amphotericin B-containing solution. A series resistance of <15 MΩ was reached 10 min following the formation of a gigaohm seal (seal resistance > 5 GΩ) and remained stable for up to 1 h. Pulse generation, data acquisition, and analysis were done with a PC equipped with a Digidata 1200 A/D interface in conjunction with Clampex 8 (Axon Instruments).

For recording V\(_m\), the extracellular medium contained 120 mM NaCl, 2 mM CaCl\(_2\), 2 mM MgCl\(_2\), 4.7 mM KCl, 0.7 mM MgSO\(_4\), 10 mM glucose, and 10 mM HEPES (pH adjusted to 7.4 with NaOH), and the pipette solution contained 50 mM KCl, 90 mM K\(^+\) aspartate, 1 mM MgCl\(_2\), and 10 mM HEPES (pH adjusted to 7.2 with KOH). The bath contained <500 µl of saline and was continuously perfused at a rate of 2 ml/min using a gravity-driven perfusion system.

**RESULTS**

**Extracellular Ca\(^{2+}\) Dependence of Basal Hormone Release**—The pattern of basal GH, PRL, and LH secretion from dispersed anterior pituitary cells was compared using rapid perfusion (1-min fractions) experiments. Basal hormone secretion was normalized to account for differences in the size of somatotroph, lactotroph, and gonadotroph populations in mixed anterior pituitary cell preparatons (Fig. 1A; see “Experimental Procedures” for details). In all of the experiments, the level of GH and PRL release was several-fold higher than that of LH release, i.e., 50–70 ng/ml for GH and PRL and below 1 ng/ml for LH. The normalized secretory profiles for each hormone from a representative experiment and the mean ± S.E. from 10 separate experiments are shown in Fig. 1B. These results demonstrate that basal GH and PRL secretion from perfused anterior pituitary cells is ~25- and 40-fold higher, respectively, than LH secretion.

To investigate the involvement of voltage-gated Na\(^+\) and Ca\(^{2+}\) channels in controlling basal GH, PRL, and LH secretion, we used blockers of these channels. Application of the specific voltage-gated Na\(^+\) channel blocker, TTX (1 µM), did not alter the pattern of basal GH, PRL, or LH secretion (Fig. 2A), indicating that these channels are not involved in the regulation of basal pituitary hormone secretion. In contrast, application of the L-type calcium channel blocker, nifedipine, and the non-specific Ca\(^{2+}\) channel blocker, Cd\(^{2+}\), inhibited basal GH and PRL secretion but did not alter basal LH secretion (Fig. 2B and C). Similarly, extracellular Ca\(^{2+}\) removal abolished the basal PRL secretion without affecting the pattern of basal LH secretion (Fig. 2D). These results indicate that the main fraction of basal GH and PRL secretion from perfused anterior pituitary cells is due to regulated, Ca\(^{2+}\)-dependent exocytosis in response to VGCs. The residual, Ca\(^{2+}\)-independent GH and PRL secretion, as well as total basal LH secretion, could be due to constitutive exocytosis or nonspecific leak of hormones dur-
this, we simultaneously monitored for the cell type-specific patterns of hormone secretion. To do so, we compared the electrical membrane activity in all three hormone-secreting cell types under identical recording conditions using the perforated patch whole cell configuration. Spontaneous AP firing with a frequency of ~0.3 Hz was observed in a majority (>80%) of the somatotrophs and lactotrophs examined (Fig. 3A). In contrast, half of the gonadotrophs examined exhibited spontaneous AP firing with a frequency of 0.7 Hz (Fig. 3A). To test whether the lower percentage of gonadotrophs exhibiting spontaneous electrical activity accounts for the low levels of basal LH secretion compared with that of GH and PRL, we increased the percentage of gonadotrophs firing APs by the addition of 5 mM K+ to 4.7 mM K+-containing M199. Potassium-induced membrane depolarization increased spike frequency in spontaneously active gonadotrophs (Fig. 3B, left traces) but did not alter the profile of the AP wave form (Fig. 3C, left traces). In addition, K+–induced membrane depolarization initiated firing in all quiescent gonadotrophs examined (Fig. 3B, right traces). These changes in Vm were accompanied with a small (less than 100 nV) increase in [Ca2+], (Fig. 3B, bottom traces). Despite the changes in the pattern of AP firing, application of 5 mM K+ did not trigger LH secretion, whereas it increased GH and PRL secretion in the same fractions. Moreover, the level of LH secretion remained lower than that of both GH and PRL secretion (Fig. 3C, right panel).

In further experiments, we examined whether differences in the ionic mechanisms of AP firing (Ca2+-dependent versus Na+-dependent spiking), the profile of the AP wave form, and/or the capacity of AP firing to drive extracellular Ca2+ entry accounts for the cell type-specific patterns of hormone secretion. To do this, we simultaneously monitored Vm activity and [Ca2+]i, in all three cell types under identical recording conditions. In spontaneously active somatotrophs and lactotrophs, extracel-

Fig. 1. Characterization of basal GH, PRL, and LH release in perifused pituitary cells from postpuberal female rats. A, percentage of immunoreactive GH-, PRL-, and LH-positive cells in mixed cultures. The values are the means ± S.E. from four experiments. B, basal hormone secretion in perifused cells. The graphs illustrate typical patterns of secretion, and the numbers below the graphs are the means ± S.E. from 10 independent experiments. In this and the following figures, secretion was analyzed in cells perifused at flow rate of 0.8 ml/min, and basal secretion was normalized to account for a difference in the size of somatotroph, lactotroph, and gonadotroph populations (see “Experimental Procedures”).

Excitability of Pituitary Cells and Basal Secretion—The involvement of VGCCs in regulating GH and PRL secretion, but not LH secretion, could be due to the inability of gonadotrophs to fire spontaneous APs. To test this, we compared the electrical membrane activity in all three hormone-secreting cell types under identical recording conditions using the perforated patch whole cell configuration. Spontaneous AP firing with a frequency of ~0.5 Hz was observed in a majority (>80%) of the somatotrophs and lactotrophs examined (Fig. 3A). In contrast, half of the gonadotrophs examined exhibited spontaneous AP firing with a frequency of 0.7 Hz (Fig. 3A). To test whether the lower percentage of gonadotrophs exhibiting spontaneous electrical activity accounts for the low levels of basal LH secretion compared with that of GH and PRL, we increased the percentage of gonadotrophs firing APs by the addition of 5 mM K+ to 4.7 mM K+-containing M199. Potassium-induced membrane depolarization increased spike frequency in spontaneously active gonadotrophs (Fig. 3B, left traces) but did not alter the profile of the AP wave form (Fig. 3C, left traces). In addition, K+–induced membrane depolarization initiated firing in all quiescent gonadotrophs examined (Fig. 3B, right traces). These changes in Vm were accompanied with a small (less than 100 nV) increase in [Ca2+], (Fig. 3B, bottom traces). Despite the changes in the pattern of AP firing, application of 5 mM K+ did not trigger LH secretion, whereas it increased GH and PRL secretion in the same fractions. Moreover, the level of LH secretion remained lower than that of both GH and PRL secretion (Fig. 3C, right panel).

In further experiments, we examined whether differences in the ionic mechanisms of AP firing (Ca2+-dependent versus Na+-dependent spiking), the profile of the AP wave form, and/or the capacity of AP firing to drive extracellular Ca2+ entry accounts for the cell type-specific patterns of hormone secretion. To do this, we simultaneously monitored Vm activity and [Ca2+]i, in all three cell types under identical recording conditions. In spontaneously active somatotrophs and lactotrophs, extracel-

Fig. 2. Extracellular calcium dependence of basal hormone release in perifused pituitary cells. A, the lack of effects of TTX, a specific blocker of voltage-gated Na+ channels, on basal hormone secretion. B, inhibition of basal hormone secretion by nifedipine, an L-type Ca2+ channel blocker. C, effects of Cd2+, a nonselective VGCC-blocker, on basal secretion. D, effects of removal of extracellular Ca2+ on basal hormone secretion. The cells were perifused with Ca2+-deficient medium containing 100 μM EGTA.

lular Ca2+ removal abolished AP firing and markedly decreased [Ca2+]i (Fig. 4, left and center traces). In spontaneously active gonadotrophs, extracellular Ca2+ removal also abolished AP firing but had only a minor effect on the already low levels of basal [Ca2+]i (Fig. 4, right traces). Thus, although all three cell types fired Ca2+-dependent APs, their capacity to drive extracellular Ca2+ entry is greater in somatotrophs and lactotrophs than in gonadotrophs.

We next examined whether differences in the profile of the AP wave form account for the cell type-specific AP-driven Ca2+ signals. Somatotrophs and lactotrophs fired low amplitude, plateau-bursting APs with a duration at one-half the amplitude of ~50 ms. The two patterns of AP firing, plateau-bursting versus single spiking, had different capacities to drive extracellular Ca2+ influx via VGCCs. The spontaneous plateau-bursting in somatotrophs and lactotrophs generated high amplitude [Ca2+]i signals that ranged from 0.3 to 1.2 μM, whereas spontaneous single spiking in gonadotrophs generated low amplitude [Ca2+]i signals ranging from 20 to 70 nM (Fig. 5).

To test whether the AP duration alone accounts for their different capacities to drive Ca2+ influx, somatotrophs, lactotrophs, and gonadotrophs were depolarized to ~10 mV for variable times (from 25 ms to 2 s), and the accompanying
increase in [Ca$^{2+}$], was monitored (Fig. 6A). In all three hormone-secreting cell types, the peak amplitude in the [Ca$^{2+}$] increased progressively with an increase in the duration of the depolarizing membrane potential step. A similar increase in the peak [Ca$^{2+}$], was observed between somatotrophs and gonadotrophs, whereas a lower [Ca$^{2+}$] response was observed in lactotrophs (Fig. 6, B–D). Nevertheless, these results indicate that the duration of VGCC alone accounts for the cell type-specific patterns of AP-driven Ca$^{2+}$ signaling.

Dependence of Basal Hormone Release on the Pattern of Firing—Our results indicate that the prolonged duration of the AP wave form in somatotrophs and lactotrophs account for the high amplitude [Ca$^{2+}$], signals and the high levels of basal hormone secretion. To test whether an increase in the duration of the AP wave form in spontaneously active gonadotrophs can increase AP-driven Ca$^{2+}$ entry and stimulate LH secretion, we used the L-type Ca$^{2+}$ channel agonist, Bay K 8644. In spontaneously active somatotrophs and lactotrophs, the addition of 1 $\mu$M Bay K 8644 increased the frequency of firing (Fig. 7A, upper traces) and the base-line [Ca$^{2+}$], (Fig. 7, A and B, bottom traces). In addition, Bay K 8644 application increased GH and PRL secretion (Fig. 7C). In spontaneously active gonadotrophs, Bay K 8644 increased the frequency of spiking and the duration of the AP wave form (Fig. 7B, e versus f). These changes in the pattern of AP firing elevated [Ca$^{2+}$], to the levels observed in unstimulated somatotrophs and lactotrophs (Fig. 7A, dashed line). Moreover, the Bay K 8644-induced increase in AP-driven Ca$^{2+}$ entry was sufficient to trigger calcium-dependent LH secretion. As shown in Fig. 7C, Bay K 8644-induced LH secretion was comparable with that observed in untreated somatotrophs and lactotrophs (dashed line). These results indicate that basal pituitary hormone secretion is dependent on the duration of the AP wave form, which determines their capacity to drive Ca$^{2+}$ entry through VGCCs.

Steady-state Depolarization and Secretion—We next compared the capacity of VGCC to stimulate hormone secretion in
each hormone-secreting cell type. To do this, we examined whether the levels of \([Ca^{2+}]_i\) and hormone secretion in response to steady-state \(V_m\) depolarization were similar between the three cell types. In gonadotrophs, \(K^+\)-induced \(V_m\) depolarization stimulated a dose-dependent increase in LH secretion (Fig. 8A). Similar results were observed in somatotrophs and lactotrophs (data not shown). Sustained membrane depolarization in all three cell types by addition of 50 mM \(K^+\) evoked a similar rise in \([Ca^{2+}]_i\) (Fig. 8B). Moreover, the normalized secretory response to 50 mM \(K^+\) was comparable in all three hormone-secreting cell types (Fig. 8C). These results argue against the hypothesis that secretory vesicles in gonadotrophs are less sensitive to \(\text{VGCC}\) compared with that in somatotrophs and lactotrophs. They also suggest that secretory vesicles in lactotrophs are more sensitive to \(\text{VGCC}\) compared with somatotrophs, because the \([Ca^{2+}]_i\) response to \(V_m\) depolarization in lactotrophs was consistently smaller than that in somatotrophs and lactotrophs (Figs. 6C and 8B), whereas basal (Figs. 1 and 2) and \(K^+\)-induced (Fig. 8C) PRL secretion was higher.

**DISCUSSION**

In this study, we examined the ionic mechanisms underlying the different patterns of basal hormone secretion from anterior pituitary somatotrophs, lactotrophs, and gonadotrophs. In general, unstimulated cells secrete in a constitutive and regulated manner, the latter through AP-driven \(Ca^{2+}\) influx and \(Ca^{2+}\)-dependent exocytosis (29–32). Our results using perifused anterior pituitary cells indicated that basal GH and PRL secretion was much higher than basal LH secretion. As in other studies (3, 4, 9), the majority of basal GH and PRL secretion was extracellular \(Ca^{2+}\)-dependent and sensitive to blockade of \(\text{VGCC}\) through \(\text{L-type channels}\). On the other hand, extracellular \(Ca^{2+}\) removal or \(\text{VGCC}\) blocker did not alter basal LH release. Basal hormone secretion from all three cell types was unaffected by the voltage-gated \(Na^{+}\) channel blocker, TTX. These results indicate that \(Ca^{2+}\)-influx via \(\text{VGCCs}\) accounts for basal GH and PRL secretion.
traces of 50 mM K

gonadotrophs. The already containing 4.7 mM K

initiate LH release. Thus, although spontaneous AP firing was frequency of firing in spontaneously active cells but did not initiate LH release. Thus, although spontaneous AP firing was observed in all three-cell types, only GH and PRL were depend-

data argue against the hypothesis that the lack of spontaneous excitability of gonadotrophs accounts for low basal LH release. Also, basal LH secretion was not related to the number of cells exhibiting spontaneous firing of APs nor to the frequency of spontaneous firing, because depolarization of cells with 5 mM K

initiated firing in quiescent gonadotrophs and increased frequency of firing in spontaneously active cells but did not initiate LH release. Thus, although spontaneous AP firing was observed in all three-cell types, only GH and PRL were depend-

The ability of AP to trigger secretion depends, in part, on the distance between secretory vesicles and VGCCs. In synapses, preocked release-ready vesicles are molecularly linked to calcium channels (33, 34), which facilitates their rapid release in response to VGCI (35). In contrast, single APs trigger only a minor amount of secretion in chromaffin cells, whereas a pro-longed step depolarization induces massive secretion that persists after VGCI has stopped (36). In rat melanotrophs, the distance between secretory vesicles and VGCCs is also large. As a result, short (40 ms) depolarizations evoked only a minor amount of secretion (37). Single cell secretory studies, using capacitance measurements, in male rat gonadotrophs also indicate that short Vm depolarizations are insufficient to stimu-

These experiments raised the possibility that secretory vesicles in somatotrophs, lactotrophs, and gonadotrophs differ in their sensitivity to VGCI, i.e. that secretory vesicles in somatotrophs and lactotrophs are close to VGCCs, whereas in gonadotrophs the localized VGCI cannot reach them. However, the results shown here indicate the opposite. The [Ca

response to square depolarizing pulses in duration of 50 ms to 2 s were comparable in the three cell types. This is in accord with earlier published results indicating that L-type Ca

channel density is similar among the three cell types (27). Furthermore, gonadotrophs, lactotrophs, and somatotrophs exhibited comparative [Ca

secretory responses during steady-state depolarization of cells with 50 mM K

, indicating that the secretory vesicles in gonadotrophs, as in somatotrophs and lactotrophs, respond to high amplitude VGCI signals. Therefore, like chromaffin cells and melanotrophs, all three anterior pituitary cell types require global [Ca

signaling to trigger substantial exocytosis.

Activation of exocytosis in unstimulated cells appears to be determined by the duration of AP wave form and its capacity to drive global Ca

signals. Somatotrophs and lactotrophs exhibit plateau-bursting activity, which leads to prolong activation of L-type channels, and sustain Ca

influx and hormone secretion. On the other hand, gonadotrophs fire single APs with a limited capacity to elevate [Ca

], and stimulate hormone secretion. A shift in the firing pattern induced by Bay K 8644, from single spiking to plateau AP accompanied with an increase in the frequency of firing in gonadotrophs was sufficient to trigger LH secretion. Although the AP duration in gonadotrophs stimulated with Bay K 8644 was shorter compared with that of plateau-bursting in somatotrophs and lactotrophs, when combined with an increase in the firing frequency it was adequate to elevate [Ca

], and LH secretion to the levels observed in unstimulated somatotrophs and lactotrophs. It should be noted, however, that an increase in spike frequency alone was not sufficient to stimulate LH secretion, as demonstrated by the inability of 5 mM K

-induced depolarization to stimulate exocytosis in gonadotrophs. In line with this, it has been shown that AP broadening contributes to the frequency-dependent facilitation of [Ca

], signals in pituitary nerve terminals (31).

The ability of somatotrophs and lactotrophs to fire low amplitude plateau-bursting type of APs and gonadotrophs to fire high amplitude single spikes indicates the cell type-specific expression of plasma membrane channels. In general, a similar group of ionic channels are expressed in each cell type, including transient and sustained VGCCs, TTX-sensitive Na

channels, transient and delayed rectifying K

channels, and multiple Ca

-sensitive K

channel subtypes (3, 8, 18, 27, 38–46). In accordance with the above hypothesis, there were marked differences in the expression levels of some of the ionic channels when analyzed in the same preparation. Specifically, lactotrophs and somatotrophs exhibited low expression levels of TTX-sensitive Na

channels and high expression levels of the
large conductance, Ca^{2+}-activated K$^+$ channel compared with those observed in gonadotrophs. In addition, functional expression of the transient K$^+$ channel was much higher in lactotrophs and gonadotrophs than in somatotrophs. The expression of the transient VGCCs was also higher in somatotrophs than in lactotrophs and gonadotrophs (27). Within these channels, it appears that BK channel activation in somatotrophs prolongs membrane depolarization, leading to the generation of plateau-bursting activity and facilitated Ca^{2+} entry. Such a paradoxical role of BK channels is determined by their rapid activation by domain Ca^{2+}, which truncates the AP amplitude and thereby limits the participation of delayed rectifying K$^+$ channels during membrane repolarization. Conversely, pituitary gonadotrophs express relatively few BK channels and fire single spikes with a low capacity to promote Ca^{2+} entry. Elevation in BK channel expression in a gonadotroph model system converted single spiking activity into plateau-bursting activity that had a high capacity to drive Ca^{2+} entry (47).

The cell type-specific AP secretion coupling observed here is consistent with hypothalamic control of pituitary hormone secretion in vivo. Initially, it was believed that all anterior pituitary cell types were under dual hypothalamic control by stimulatory and inhibitory factors. This remains true for somatotrophs and lactotrophs, in which the dual hypothalamic control of GH and PRL secretion is well established (reviewed in Refs. 48 and 49). A negative hypothalamic factor controlling gonadotropin secretion, however, has not been identified. Moreover, the data shown here confirm that there is no need for such regulation, because basal LH secretion is very low. The dual control of somatotrophs and lactotrophs is essential for generating the episodic release of GH and PRL (48, 49), whereas the work by Knobil (50) and others (51) has established that the hypothalamic GnRH pulse generator itself accounts for the pulsatile release of LH. Furthermore, episodic LH release is required for normal reproductive functions, and AP secretion coupling in spontaneously active gonadotrophs, like continuous GnRH administration (50), would inhibit the reproductive cycle.

The lack of AP-induced secretion in unstimulated gonadotrophs does not diminish the importance of AP firing in these cells. Although subthreshold for activation of exocytosis, spontaneous VGCC in gonadotrophs maintain the [Ca^{2+}]_{i} at the optimal level required for interactions between inositol (1,4,5)-trisphosphate and Ca^{2+} in their dual control of inositol (1,4,5)-trisphosphate channel gating (52, 53). Furthermore, GnRH-induced and inositol (1,4,5)-trisphosphate-mediated [Ca^{2+}], oscillations in gonadotrophs generate transient \( V_m \) hyperpolarizations, upon which bursting firing is observed (23, 24). Although the agonist-induced shift in the pattern of AP firing alone cannot protect against depletion of the intracellular Ca^{2+} stores, it provides a steady-state increase in VGCC during prolonged GnRH stimulation (53). Combined with the redistribution of Ca^{2+} between mitochondria and endoplasmic reticulum (54), such VGCC is sufficient to maintain agonist-induced [Ca^{2+}], oscillations and LH release for several hours (19).

In conclusion, our results indicate that spontaneous, extracellular Ca^{2+}-dependent AP firing is a common feature of pituitary somatotrophs, lactotrophs, and gonadotrophs. Such \( V_m \) oscillations were sufficient to stimulate GH and PRL but not LH release. This indicates that cell excitability per se is not sufficient for an effective AP secretion coupling in excitable endocrine cells as it is in neuronal cells during synaptic transmission. Our results further indicate that the pattern of spontaneous electrical activity encodes the cell type-specific basal hormone secretion. Specifically, somatotrophs and lactotrophs fire plateau-bursting APs with a high capacity to drive Ca^{2+} entry, whereas gonadotrophs fire single spikes with a low capacity to drive Ca^{2+} entry. The cell type-specific AP secretion coupling in pituitary cells described here provides a rationale for the existence of negative hypothalamic control of PRL and GH but not LH secretion.

**REFERENCES**

1. Stojilkovic, S. S., Izumi, S.-I., and Catt, K. J. (1988) J. Biol. Chem. **263**, 10554–10561.
2. Holl, R. W., Thorner, M. O., and Leong, D. A. (1989) Am. J. Physiol. **256**, E573–E579.
3. Cota, G., Hiriart, M., Horta, J., and Torres-Escalante, J. L. (1990) Am. J. Physiol. **259**, C949–C959.
4. Enyeart, J. J., Sheu, S. S., and Hinkle, P. M. (1987) J. Biol. Chem. **262**, 3154–3159.
5. Marz, D., Simonovic, I., Kovacevic, R., Krsmanovic, L., Stojilkovic, S., and Andjus, R. K. (1982) J. Endocrinol. Invest. **5**, 235–241.
6. Stojilkovic, S. S., and Catt, K. J. (1992) Endocr. Rev. **13**, 256–280.
7. Kehl, S. J., and Wong, K. (1996) *J. Membr. Biol.* **141**, 491–499.
8. Sims, S. M., Lussier, B. T., and Kraicer, J. (1998) J. Physiol. **510**, 615–637.
9. Holl, R. W., Thorner, M. O., Mandell, G. L., Sullivan, J. A., Sinha, Y. N., and Leong, D. A. (1988) J. Biol. Chem. **263**, 9682–9685.
10. Simasko, S., M., and Simasko, S. M. (1996) Am. J. Physiol. **271**, C1927–C1934.
11. Ingram, C. D., Bicknell, R. J., and Mason, W. T. (1986) *Endocrinology* **119**, 1536–1545.
12. Biales, B., Dicher, M. A., and Tischler, A. (1977) *Nature* **275**, 172–174.
13. KidoKoro, Y. (1975) *Nature* **258**, 741–742.
14. Dors, R., Vincent, J. D., Fleury, L., Da Pasquier, P., Gourdiard, D., and Tizier, Vidal, A. (1979) *Science* **204**, 309–311.
15. Schlegel, W., Winiger, B. P., Mollard, P., Vacher, P., Wuarin, F., Zahnd, G. R., Wolfheim, C. B., and Dufy, B. (1987) *Nature* **329**, 719–721.
16. Kvisicin, R., Robert, C., Cano, R., Viguier, S., Arnoux, A., Kordon, C., and Hammond, C. (1998) *Physiol. Rev.* **78**, 883–905.
17. Bonnefont, X., Fiekers, J., Creff, A., and Mollard, P. (2000) *Endocrinology* **141**, 865–878.
18. Tse, A., and Hille, B. (1993) *Endocrinology* **132**, 1475–1481.
19. Stojilkovic, S. S., Kukuljan, M., Iida, T., Rojas, E., and Catt, K. J. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4081–4085.
20. Tse, P. W., Tse, A., Hille, B., and Almers, W. (1997) *Nature* **383**, 121–132.
21. Kehl, S. J., and Wong, K. (1996) *J. Membr. Biol.* **150**, 219–230.
22. Koshimizu, T., Tomive, M., Wilson, A. O. L., Zivadinovic, D., and Stojilkovic, S. S. (1990) *Endocrinology* **190**, 4911–4919.
23. Kukuljan, M., Stojilkovic, S. S., Rojas, E., and Catt, K. J. (1992) *FEBS Lett.* **310**, 19–22.
24. Tse, A., and Hille, B. (1992) *Science* **255**, 462–464.
25. Chang, J. P., Stojilkovic, S. S., Graeter, J. S., and Catt, K. J. (1988) *Endocrinology* **123**, 87–97.
26. Rae, J., Cooper, K., Gates, P., and Watsky, M. (1991) *J. Neurosci. Methods* **37**, 15–26.
27. Van Goor, F., Zivadinovic, D., and Stojilkovic, S. S. (2001) *Mol. Endocrinol.* **15**, 1222–1236.
28. von Ruden, L., and Neher, E. (1993) *Science* **262**, 1061–1064.
29. Manohar, H. R., and Catterall, W. A. (1989) *J. Biol. Chem.* **264**, 7304–7308.
30. Lim, N. F., Nowycky, C. M., and Bookman, R. J. (1990) *Nature* **344**, 449–451.
31. Stanley, E. F. (1986) *J. Neurosci.* **6**, 782–789.
32. Zheng, X., Betting, J., Cook, T., and Catterall, W. A. (1996) *Nature* **379**, 451–454.
33. Schaffer, Z., Ursenbach, S., Innes, K., and Innes, R. (2006) *J. Neurosci.* **26**, 10330–10339.
Dependence of Pituitary Hormone Secretion on the Pattern of Spontaneous
Voltage-gated Calcium Influx: CELL TYPE-SPECIFIC ACTION POTENTIAL
SECRETION COUPLING
Fredrick Van Goor, Dragoslava Zivadinovic, Antonio J. Martinez-Fuentes and Stanko S.
Stojilkovic

J. Biol. Chem. 2001, 276:33840-33846.
doi: 10.1074/jbc.M105386200 originally published online July 16, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M105386200

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 53 references, 13 of which can be accessed free at
http://www.jbc.org/content/276/36/33840.full.html#ref-list-1