Differential Expression of Ionic Channels in Rat Anterior Pituitary Cells

Fredrick Van Goor, Dragoslava Zivadinovic, and Stanko S. Stojilkovic

Endocrinology and Reproduction Research Branch
National Institute of Child Health and Human Development
National Institutes of Health
Bethesda, Maryland 20892-4510

Secretory anterior pituitary cells are of the same origin, but exhibit cell type-specific patterns of spontaneous intracellular Ca\(^{2+}\) signaling and basal hormone secretion. To understand the underlying ionic mechanisms mediating these differences, we compared the ionic channels expressed in somatotrophs, lactotrophs, and gonadotrophs from randomly cycling female rats under identical cell culture and recording conditions. Our results indicate that a similar group of ionic channels are expressed in each cell type, including transient and sustained voltage-gated Ca\(^{2+}\) channels, tetrodotoxin-sensitive Na\(^{+}\) channels, transient and delayed rectifying K\(^{+}\) channels, and multiple Ca\(^{2+}\)-sensitive K\(^{+}\) channel subtypes. However, there were marked differences in the expression levels of some of the ionic channels. Specifically, lactotrophs and somatotrophs exhibited low expression levels of tetrodotoxin-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. Finally, the expression of the transient voltage-gated Ca\(^{2+}\) channels was higher in lactotrophs and gonadotrophs than in somatotrophs. These results indicate that there are cell type-specific patterns of ionic channel expression, which may be of physiological significance for the control of Ca\(^{2+}\) homeostasis and secretion in unstimulated and receptor-stimulated anterior pituitary cells. (Molecular Endocrinology 15: 1222–1236, 2001)

INTRODUCTION

The anterior pituitary is composed of the five major hormone-secreting cell types, corticotrophs, lactotrophs, thyrotrophs, somatotrophs, and gonadotrophs. Corticotrophs arise from a lineage that is distinct from the other cell types, whereas the remaining cell types share common transcription factors and frequently produce multiple hormones, indicating that they are closely related (1). Despite their similar origin, somatotrophs, lactotrophs, and gonadotrophs differ with respect to their pattern of spontaneous electrical activity, intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]) signaling, basal hormone secretion, and neuroendocrine regulation of spontaneous Ca\(^{2+}\) influx and Ca\(^{2+}\)-dependent hormone secretion. Specifically, somatotrophs and lactotrophs exhibit extracellular Ca\(^{2+}\)-dependent, high-amplitude [Ca\(^{2+}\)], transients, whereas only low-amplitude [Ca\(^{2+}\)], signals have been observed in unstimulated gonadotrophs (2, 3). In parallel to spontaneous [Ca\(^{2+}\)] signaling, somatotrophs and lactotrophs exhibit high basal secretion, whereas basal gonadotropin secretion is low and not dependent on extracellular Ca\(^{2+}\) (4). Consistent with this, Ca\(^{2+}\) signaling and secretion in somatotrophs and lactotrophs, but not in gonadotrophs, are under dual control by positive and negative hypothalamic factors (5–7). These differences suggest that, despite the similar origin of somatotrophs, lactotrophs, and gonadotrophs, there may be differences in the ionic channels expressed in the three hormone-secreting cell types.

Many of the ionic channels in native and immortalized anterior pituitary cells have been characterized previously, including voltage-gated Ca\(^{2+}\) channels (VGCCs), tetrodotoxin (TTX)-sensitive and insensitive Na\(^{+}\) channels, voltage-gated K\(^{+}\) channels, Ca\(^{2+}\)-controlled K\(^{+}\) channels, Cl\(^{-}\) channels, and nonselective cationic channels (2, 3). However, it is difficult to directly compare the expression levels and the properties of the individual ionic channels from the different studies, due to differences in the species, sex, and hormonal status of the animals used, as well as the cell
cultures and recording conditions. Because of this, it is still not known whether differences in the expression levels and/or ionic channel properties underlie the cell type-specific patterns of voltage-gated Ca\(^{2+}\) entry and hormone secretion. To address this problem, we compared the expression levels and voltage-dependent properties of the ionic channels in somatotrophs, lactotrophs, and gonadotrophs from randomly cyclic female rats under identical culture and recording conditions. A similar group of ionic channels is observed in all three cell types. However, there was a marked difference in the functional expression of the individual ionic channels between the three hormone-secreting cell types. These differences likely underlie the cell type-specific patterns of [Ca\(^{2+}\)]\(_i\) signaling and hormone secretion observed in unstimulated somatotrophs, lactotrophs, and gonadotrophs.

RESULTS

Voltage-Gated Na\(^{+}\) Channels

The functional expression and voltage-dependent properties of the Na\(^{+}\) channels in rat somatotrophs, lactotrophs, and gonadotrophs were examined under isolated Na\(^{+}\) current (\(I_{\text{Na}}\)) recording conditions using the conventional whole-cell technique. In rat somatotrophs and lactotrophs, a rapidly activating and inactivating \(I_{\text{Na}}\) was observed at membrane potentials more depolarized than \(-37\) mV and reached maximum amplitude around \(-7\) mV (Fig. 1, A, B, and D). A similar \(I_{\text{Na}}\) was observed in rat gonadotrophs (Fig. 1C), but its peak amplitude was much greater and its current-voltage relation was shifted approximately \(10\) mV in the hyperpolarizing direction compared with that for somatotrophs and lactotrophs.

**Fig. 1.** Voltage-Gated Na\(^{+}\) Channels in Somatotrophs, Lactotrophs, and Gonadotrophs

Representative voltage-gated \(I_{\text{Na}}\) traces in somatotrophs (panel A, \(n = 5\)), lactotrophs (panel B, \(n = 5\)), and gonadotrophs (panel C, \(n = 5\)) elicited by 100-msec voltage steps from \(-87\) mV to \(83\) mV form a holding potential of \(-117\) mV. D, Current-voltage relation of the voltage-gated \(I_{\text{Na}}\) in all three cell types. E, Steady-state inactivation curves for the voltage-gated \(I_{\text{Na}}\) in all three cell types were generated by stepping the membrane potential to between \(-127\) and \(-13\) mV for 200 msec before stepping to a 100-msec command potential of \(-17\) mV (holding potential = \(-97\) mV). The peak \(I_{\text{Na}}\) evoked during the command potential to \(-17\) mV in each cell type were normalized to the maximum inward current and plotted against the conditioning pulse potentials. In this and the following figures, the ionic currents were normalized to the membrane capacitance of each cell examined to compensate for the differences in the size of cells. The dashed box in this figure and in Figs. 3 and 5 represents the range of baseline potentials commonly observed in spontaneously active somatotrophs, lactotrophs, and gonadotrophs (54).
somatotrophs and lactotrophs (Fig. 1D). In somatotrophs, lactotrophs, and gonadotrophs, application of 1 μM TTX reduced the peak $I_{Na}$ amplitude by 99.4 ± 0.7% ($n = 5$), 98.2 ± 3.3% ($n = 5$), and 98.9 ± 1.2% ($n = 5$), respectively.

To determine the proportions of the TTX-sensitive $I_{Na}$ in each cell type that are available for activation at different resting membrane potentials, the steady-state inactivation properties of the $I_{Na}$ in all three cell types were examined using a two-pulse protocol. This protocol consisted of a series of 200-msec conditioning pulses from $-127$ mV to $-13$ mV, followed by a 100-msec test pulse to $-17$ mV (holding potential = $-97$ mV). The peak $I_{Na}$ evoked during the test pulse was normalized to the maximal inward current and plotted against the conditioning pulse potentials, and the resulting curves were fitted with a single Boltzmann relation (Fig. 1E). In somatotrophs, the membrane potential at which there is 50% of the maximal current ($E_{1/2}$) available for activation is $-72$ mV. In lactotrophs and gonadotrophs, the $E_{1/2}$ values were similar and were approximately 10 mV more depolarized than that observed in somatotrophs.

**Voltage-Gated Ca$^{2+}$ Channels**

The functional expression and voltage-dependent properties of the Ca$^{2+}$ channels in rat somatotrophs, lactotrophs, and gonadotrophs were examined under isolated Ca$^{2+}$ current ($I_{Ca}$) recording conditions using the conventional whole-cell technique. The $I_{Ca}$ in each cell type was examined by applying a series of 400-msec depolarizing voltage steps from $-97$ mV to $+83$ mV in 10-mV increments (holding potential = $-97$ mV). In all three cell types, a transient $I_{Ca}$ was observed in response to the depolarizing voltage steps. In somatotrophs, the transient $I_{Ca}$ was activated by voltage steps more depolarized than $-77$ mV and reached maximum amplitude between $-27$ and $-17$ mV (Fig. 2, A and B). In the other two cell types, the activation threshold of the transient current was shifted approximately 10 mV in the depolarizing direction (Fig. 2, A and B). In addition, the peak of the transient $I_{Ca}$-voltage relationship was shifted more than 20 mV in the depolarizing direction compared with that in somatotrophs (Fig. 2B). To determine the density of the transient $I_{Ca}$ in each cell type, the peak $I_{Ca}$ was normalized to the maximal inward current and plotted against the membrane potential at $-97$ mV.
I_{\text{ca}} evoked by voltage steps to −47 mV (holding potential = −97 mV) was analyzed. This membrane potential was used because it activates a transient I_{\text{ca}} in all three cell types with only minimal activation of a sustained I_{\text{ca}} (Fig. 2A). The mean peak transient I_{\text{ca}} densities at −47 mV in somatotrophs, lactotrophs, and gonadotrophs were −6.6 ± 0.8 picoamperes/picofarads (pA/pF) (n = 20), −2.2 ± 0.3 pA/pF (n = 16), and −2.7 ± 0.6 pA/pF (n = 14), respectively.

The transient I_{\text{ca}} in somatotrophs, lactotrophs, and gonadotrophs was followed by slow-inactivating I_{\text{ca}}. In all three cell types, the sustained I_{\text{ca}} was activated by voltage steps more depolarized than −57 mV and reached a maximum amplitude around +3 mV (Fig. 2, A and B). In addition to the similar current-voltage relationship of the sustained I_{\text{ca}}, dihydropyridine agonists and antagonists had a similar effect on the sustained I_{\text{ca}} amplitude in all three cell types (Table 1). To compare the density of the slow-inactivating I_{\text{ca}} in each cell type, the sustained I_{\text{ca}} (390–400 msec) evoked by voltage steps to +3 mV was analyzed. In somatotrophs, lactotrophs, and gonadotrophs the mean sustained I_{\text{ca}} densities were −6.8 ± 0.8 pA/pF (n = 20), −4.5 ± 0.9 pA/pF (n = 16), and −9.2 ± 1.7 pA/pF (n = 14), respectively.

To determine the proportions of the total voltage-gated I_{\text{ca}} in each cell type that is available for activation at various different resting membrane potentials, the steady-state inactivation properties of the I_{\text{ca}} were examined using a two-pulse protocol. This protocol consisted of a conditioning pulse ranging from −127 mV to −13 mV for 400 msec, after which a 200-msec test pulse to −7 mV was applied (Fig. 3, A–C). The normalized test current in each cell type was plotted against the conditioning pulse potentials, and the resulting curve was fitted with a single Boltzmann relation (Fig. 3D). In somatotrophs, E_{1/2} was −56 mV (slope factor = 6.5). The E_{1/2} in lactotrophs and gonadotrophs were −34 mV (slope factor = 7) and −40 mV (slope factor = 4), respectively, which were more than 15 mV more depolarized than that in somatotrophs. The more pronounced steady-state inactivation of the total I_{\text{ca}} in somatotrophs compared with that in lactotrophs and gonadotrophs is consistent with the greater expression of the transient I_{\text{ca}} in somatotrophs.

Voltage-Gated K⁺ Channels

The functional expression and voltage-dependent properties of the voltage-gated K⁺ channels in rat somatotrophs, lactotrophs, and gonadotrophs were examined under isolated K⁺ current (I_{\text{K}}) recording conditions using the perforated-patch technique. To exclude Ca²⁺-sensitive I_{\text{K}} (I_{\text{K(Ca)}}), extracellular Ca²⁺ entry through VGCCs was blocked by addition of 200 μM Cd²⁺ to the bath solution. The total voltage-gated I_{\text{K}} in each cell type was examined by the application of a 500-msec holding potential to −130 mV before giving a series of 1.5-sec depolarizing voltage steps from −90 mV to +90 mV (Fig. 4A; upper panels). In rat somatotrophs, both the peak (0–25 msec) and sustained (1.4–1.5 sec) I_{\text{K}} were activated at membrane potentials more depolarized than −30 mV. Once activated, the total voltage-gated I_{\text{K}} in each cell type was examined by the application of a 500-msec holding potential to −130 mV before giving a series of 1.5-sec depolarizing voltage steps (Fig. 4, A and D). In lactotrophs and gonadotrophs, the peak I_{\text{K}} activated at membrane potentials more depolarized than −50 mV, whereas the sustained I_{\text{K}} activated at membrane potentials more depolarized than −30 mV. Unlike somatotrophs, the total voltage-gated I_{\text{K}} in lactotrophs and gonadotrophs was characterized by a fast- and slow-inactivating I_{\text{K}} (Fig. 4, A and D).

To isolate the slow-inactivating I_{\text{K}}, the transient I_{\text{K}} was eliminated by the application of a 500-msec holding potential to −40 mV before the 1.5-sec depolarizing voltage steps from −90 mV to +90 mV (Fig. 4B). Under these conditions, a slow-inactivating I_{\text{K}} was observed in somatotrophs and lactotrophs (Fig. 4B). A similar slow-inactivating I_{\text{K}} was observed in gonadotrophs in response to voltage steps between −40 and 20 mV. However, a small fast-inactivating I_{\text{K}} was maintained at more depolarized membrane potentials, which may be due to incomplete inactivation of the transient I_{\text{K}} in gonadotrophs by the conditioning pulse. To compare the densities of the slow-inactivating I_{\text{K}} in each cell type, the sustained I_{\text{K}} evoked by voltage steps to +90 mV (holding potential of −90 mV) was analyzed. The sustained I_{\text{K}} density in lactotrophs was 147 ± 15 pA/pF (n = 5), which was significantly (P < 0.05) smaller than that in somatotrophs (254 ± 32 pA/pF; n = 5) and gonadotrophs (282 ± 32 pA/pF; n = 8).

### Table 1. Effects of L-Type Ca²⁺ Channel Agonists and Antagonists on Sustained I_{\text{ca}} in Somatotrophs, Lactotrophs, and Gonadotrophs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Somatotrophs (pA/pF)</th>
<th>Lactotrophs (pA/pF)</th>
<th>Gonadotrophs (pA/pF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (5)</td>
<td>9.8 ± 2.0</td>
<td>4.6 ± 2.5</td>
<td>7.9 ± 2.5</td>
</tr>
<tr>
<td>1 μM Nifedipine (8)</td>
<td>6.7 ± 1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.0 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1 μM Bay K 8644 (8)</td>
<td>17.0 ± 3.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.5 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.2 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All I_{\text{ca}} were evoked by a 200-msec voltage step to −7 mV from a holding potential of −87 mV and are expressed as mean ± SEM. The peak I_{\text{ca}} between 190 and 200 msec are shown.

<sup>a</sup> Number of trials in each treatment group.

<sup>b</sup> Significant difference (P < 0.05) compared to controls.
We next compared the transient $I_K$ in each cell type by subtracting the $I_K$ elicited during the various voltage steps after a holding potential to $-240$ mV from the total $I_K$ (Fig. 4C). In rat somatotrophs, a small transient $I_K$ was evoked by membrane potential steps more depolarized than $-50$ mV and reached maximum amplitude at $+90$ mV of $343 \pm 73$ pA/pF and $389 \pm 82$ pA/pF at $+90$ mV, respectively.

To determine the proportions of the total voltage-gated $I_K$ in each cell type that is available for activation at various different membrane potentials, the steady-state inactivation properties of the $I_K$ were examined using a two-pulse protocol. This protocol consisted of a series of 1.5-sec conditioning pulses from $-130$ mV to $-10$ mV, followed by a 400-msec test pulse to $+90$ mV (Fig. 5A). The representative $I_K$ tracings evoked by the two-pulse protocol are shown in Fig. 5, A–C. The normalized test current in each cell type was plotted against the conditioning pulse potentials (Fig. 5D). A single Boltzmann relation could not be fitted to the data, further indicating the presence of a fast- and slow-inactivating component in each of the three cell types. However, the magnitude of steady-state $I_K$ inactivation in somatotrophs was much less than that observed in the other two cell types, which is consistent with the low expression levels of the transient $I_K$ in somatotrophs.

**Ca$^{2+}$-Activated K$^+$ Channels**

Voltage-gated Ca$^{2+}$ entry was used to examine the expression of $I_{K(Ca)}$ in somatotrophs, lactotrophs, and gonadotrophs. To activate VGCCs, a modified two-step protocol was used. This protocol consisted of an initial 100-msec voltage-step to $-10$ mV (holding potential $= -90$ mV) during which VGCCs were activated (Fig. 6A; left panel). This Ca$^{2+}$-influx step was immediately followed by a 500-msec test pulse to $+80$ mV, during which the evoked $I_K$ was monitored. As $+80$ mV is near the reversal potential for Ca$^{2+}$ under our experimental conditions, there should be minimal Ca$^{2+}$ entry during this step. Consistent with this, in the absence of the Ca$^{2+}$-influx step, there was little to no change in [Ca$^{2+}$], whereas in the presence of a Ca$^{2+}$-influx step alone or in combination with the test pulse there was a marked increase in [Ca$^{2+}$], in all three cell types (Fig. 7). In addition, the $I_K$ evoked by the test pulse to $+80$ mV in the absence of the Ca$^{2+}$-influx step was similar in Ca$^{2+}$-containing and Ca$^{2+}$-deficient medium (Table 2).

In the presence of a 100-ms Ca$^{2+}$-influx step, the peak $I_K$ evoked during the test pulse was reduced by extracellular Ca$^{2+}$ removal in somatotrophs, lactotrophs, and gonadotrophs (Fig. 6, A–C; and Table 3), indicating $I_{K(Ca)}$ values are expressed in all three cell types. To compare $I_{K(Ca)}$ activation between the three cell types, the $I_K$ evoked in Ca$^{2+}$-deficient medium was subtracted from that in Ca$^{2+}$-containing medium (Fig. 6, A–C, right panels). These results clearly indicate that $I_{K(Ca)}$ activation by voltage-gated Ca$^{2+}$ entry was greatest in somatotrophs and smallest in gonadotrophs.
The differences in IK(Ca) activation between the three cell types may be due to differences in the ability of the Ca$^{2+}$ influx step to drive voltage-gated Ca$^{2+}$ entry and increase [Ca$^{2+}$]. To test this, we compared the increase in [Ca$^{2+}$], evoked by the two-step protocol in each cell type (Fig. 7). In somatotrophs and gonadotrophs, the two-step protocol increased the [Ca$^{2+}$] by 160 ± 18 nm (n = 17) and 164 ± 19 nm (n = 12),
respectively. In lactotrophs, a smaller increase in 
[Ca^{2+}]_i of 122 ± 6 13 nM (n = 12) was evoked by the 
two-step protocol. These results are consistent with 
the smaller amplitude noninactivating I_{Ca} in lac-
totrophs compared with the other two cell types (Fig.

2). Nevertheless, these results indicate that the small 
amplitude I_{K(Ca)} observed in gonadotrophs is not due 
to the inability of voltage-gated Ca^{2+} entry to increase 
[Ca^{2+}]. Therefore, the differences between I_{K(Ca)} acti-
vation in the three cell types appears to be due to 
differences in channel expression and not the capacity 
of the Ca^{2+}-influx step to drive changes in [Ca^{2+}].

The dependence of I_{K(Ca)} activation on voltage-
gated Ca^{2+} influx was further confirmed in experi-
ments with dihydropyridine agonist and antagonists. 
As shown in Fig. 8, A and C, addition of the L type 
Ca^{2+} channel blocker, nifedipine, significantly de-
creased the amplitude of the I_{K} in all three cell types 
studied. Furthermore, Bay K 8644, an L-type Ca^{2+} 
channel agonist, significantly increased the amplitude 
of I_{K} in all three cell types (Fig. 8, A and C). Consistent 
with the differential expression of Ca^{2+}-activated K^{+} 
channels in the three cell types, the effects of nifedi-
and lactotrophs, but not in gonadotrophs (Fig. 9D and Table 3). Unlike the BK channel blockers, the SK channel blocker, apamin, had no effect on the $I_K$ evoked by the 100-msec Ca$^{2+}$-influx step in the majority of somatotrophs ($n = 27$) and lactotrophs ($n = 11$) examined. In the remaining somatotrophs and lactotrophs, apamin significantly reduced the $I_K$ (Fig. 9E and Table 3). In all gonadotrophs examined ($n = 10$), apamin had no effect on the $I_K$ evoked by the 100-msec Ca$^{2+}$-influx step (Fig. 9E and Table 3). This was not due to the absence of SK channels in these cells, as GnRH simulated $I_{BK}$ in all gonadotrophs (data not shown).

In the presence of apamin and CTX, IBTX, or paxilline, no further reduction in $I_K$ was observed when the cells were perfused with Ca$^{2+}$-deficient medium (data not shown). These results indicate that $I_{BK}$ activation by voltage-gated Ca$^{2+}$ influx in somatotrophs, lactotrophs, and gonadotrophs is predominantly mediated by BK channels. It should be noted, however, that the inability of apamin to significantly reduce the $I_K$ in a majority of the cells examined is most likely due to the relatively small current generated by its activation and not the lack of expression in each cell type. In addition, other Ca$^{2+}$-sensitive currents that are expressed in pituitary cells, such as Ca$^{2+}$-activated Cl$^-$ channels (8, 9), may be masked by the much larger $I_K$ in these cells.

To determine the duration of voltage-gated Ca$^{2+}$ influx required to activate $I_{BK}$ in each cell type, we varied the duration of the Ca$^{2+}$-influx step and measured the peak $I_K$ amplitude during the subsequent 500-msec voltage step to +80 mV. To isolate BK channels from SK channels, 100 nM apamin was added to the extracellular medium. In somatotrophs, 5-msec Ca$^{2+}$-influx steps were sufficient to activate $I_{BK}$, whereas 10-msec steps were required in lactotrophs. In both cell types, the peak $I_K$ increased progressively in response to incremental increases in the duration of the Ca$^{2+}$-influx step (Fig. 10, A–C). This increase was not observed in Ca$^{2+}$-deficient medium (Fig. 10, B and C) or in cells preloaded with BAPTA-AM (Fig. 10C). In gonadotrophs, Ca$^{2+}$-influx steps greater than 75 msec were required to activate $I_{BK}$ (Fig. 10, A–C). In addition, no increase in $I_{BK}$ activation was observed in response to further increases in the duration of voltage-gated Ca$^{2+}$ influx (Fig. 10C). These results suggest that brief periods of voltage-gated

### Table 2. Peak $I_K$ in Somatotrophs, Lactotrophs, and Gonadotrophs Evoked by a 500-msec Voltage Step from -90 mV to +80 mV in Ca$^{2+}$-Containing and Ca$^{2+}$-Deficient Medium

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Ca$^{2+}$-Containing (pA/pF)</th>
<th>Ca$^{2+}$-Deficient (pA/pF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatotrophs</td>
<td>306 ± 14</td>
<td>309 ± 18</td>
</tr>
<tr>
<td>Lactotrophs</td>
<td>233 ± 16</td>
<td>229 ± 26</td>
</tr>
<tr>
<td>Gonadotrophs</td>
<td>434 ± 24</td>
<td>442 ± 29</td>
</tr>
</tbody>
</table>

All currents are expressed as mean ± SEM. * Numbers in parentheses indicate number of trials in each treatment group.
Ca\textsuperscript{2+} influx are sufficient to activate BK channels in somatotrophs and lactotrophs, whereas prolonged Ca\textsuperscript{2+} influx is required in gonadotrophs. Moreover, it appears that increasing the duration of voltage-gated Ca\textsuperscript{2+} influx recruits more BK channels in somatotrophs and lactotrophs, but not gonadotrophs.

**DISCUSSION**

Despite the similar origin of somatotrophs, lactotrophs, and gonadotrophs, these cells differ with respect to their pattern of basal hormone secretion. Specifically, basal gonadotropin is low compared with...
basal GH and PRL secretion. Earlier studies have indicated that these differences in basal hormone secretion are due to differences in spontaneous Ca^{2+} signaling among the three cell types. Unlike basal GH and PRL secretion, basal gonadotropin secretion is not dependent on the presence of extracellular Ca^{2+} or modified by increases in the extracellular Ca^{2+} concentration (4). In addition, application of voltage-gated Ca^{2+} channel blockers reduces basal GH and PRL secretion, but not basal LH or FSH secretion (4, 25). Consistent with these differences in the apparent role of voltage-gated Ca^{2+} channels in controlling basal hormone secretion, the profile of spontaneous Ca^{2+} signaling among the three cell types is also different. In rat lactotrophs and somatotrophs, spontaneous and high amplitude Ca^{2+} signals have been observed, whereas only low amplitude Ca^{2+} signals have been observed in spontaneously active rat gonadotrophs (2–4, 25, 35). These differences in the extracellular Ca^{2+} dependency and voltage-gated Ca^{2+} channel involvement in determining the pattern of basal hormone secretion and the cell type-specific patterns of spontaneous Ca^{2+} signaling suggest that there are differences in the level and/or composition of ionic channels in anterior pituitary cells.

Fig. 9. Pharmacological Identification of BK Channels in Pituitary Cells

A–D, Representative current traces showing the effects of 100 nM IBTX (A), 100 nM CTX (B), 1 μM paxilline (C), 30 μM NS 1619 (D), and 100 mM apamin (E), on the I_K in somatotrophs, lactotrophs, and gonadotrophs. Averaged data for each cell type are shown in Table 3.
channel expression and the underlying electrical activity between rat gonadotrophs, lactotrophs, and somatotrophs.

In this study, we compared the expression levels of different ionic channels between pituitary somatotrophs, lactotrophs, and gonadotrophs. As a measure of the functional expression levels of the ionic channels in each cell type, the isolated currents were analyzed. The results indicate a quantitative rather than qualitative difference in the expression pattern for the major ionic currents in pituitary cells. All three cell types express transient and sustained voltage-gated Ca$^{2+}$ channels, TTX-sensitive Na$^{+}$ channels, transient and delayed rectifying K$^{+}$ channels, and multiple Ca$^{2+}$-sensitive K$^{+}$ channel subtypes. Lactotrophs and somatotrophs exhibited low expression levels of TTX-sensitive Na$^{+}$ channels and high expression levels of BK channel compared with those observed in gonadotrophs. The expression of the transient voltage-gated K$^{+}$ channel was much higher in lactotrophs and gonadotrophs than in somatotrophs. Finally, the expression of the transient VGCCs was higher in somatotrophs than in lactotrophs and gonadotrophs.

TTX-sensitive Na$^{+}$ channels have been previously identified in gonadotrophs (10, 11), lactotrophs (12), and somatotrophs (13), as well as other pituitary cell types (14–16), indicating that these channels are common among mammalian pituitary cells. Our results extended these findings by demonstrating that the level of Na$^{+}$ channel expression is much greater in rat gonadotrophs than the other two cell types. However, it is unlikely that the difference in the expression of these channels is relevant for the control of spontaneous electrical activity (12, 17), Ca$^{2+}$ signaling (17), and basal hormone secretion (16, 18) in these cells. The lack of TTX-sensitive Na$^{+}$ channel involvement in controlling membrane excitability and secretion is most likely due to the inactivation of a large proportion of these channels at the resting membrane potential in these cells (Fig. 1 and Ref. 10). Consistent with this, GnRH-induced transient membrane hyperpolarization in rat gonadotrophs is required to remove the steady-

---

**Fig. 10. Dependence of $I_{BK}$ Activation on Duration of Voltage-Gated Ca$^{2+}$ Influx**

The effects of increasing the duration of VGCC entry on $I_{BK}$ activation were examined by incremental increases in the duration of Ca$^{2+}$ influx step from 0 to 100 msec, followed by a test pulse to +80 mV for 500 msec. Representative current tracings recorded in Ca$^{2+}$-containing (A) or Ca$^{2+}$-deficient medium (B) are shown for somatotrophs (n = 13), lactotrophs (n = 12), and gonadotrophs (n = 12). C, Peak $I_{BK}$ amplitude (mean ± SEM) during the test pulse after 0- to 150-msec conditioning pulses in Ca$^{2+}$-containing medium (filled circles), Ca$^{2+}$-deficient medium (open circles), and in cells preloaded with BAPTA-AM (filled triangles). Asterisks indicate a significant difference compared with the peak $I_{BK}$ evoked in the absence of a depolarizing pulse. All experiments were performed in the presence of 100 nM apamin to block SK channel activation.
state inactivation of TTX-sensitive Na\(^+\) channels before they can contribute to action potential (AP) firing (10). This TTX sensitivity during agonist-induced AP firing in gonadotrophs is important for sustained Ca\(^{2+}\)-signaling through VGCCs and the refilling of endoplasmic reticulum Ca\(^{2+}\) stores. There are, however, two exceptions. First, in a fraction of ovine gonadotrophs, these channels are responsible for AP generation (19). Second, two lactotroph subpopulations have been identified that differ with respect to their level of Na\(^+\) channel expression, and only in lactotrophs expressing high levels of Na\(^+\) channels did TTX application abolish basal hormone secretion (20). In the present study, we did not observe a significant difference in the level of Na\(^+\) channel expression in the lactotrophs identified by our cell separation and identification protocol.

The expression of both inactivating and noninactivating VGCCs in rat gonadotrophs (10, 21, 22), somatotrophs (13), and lactotrophs (13), as well as immortalized pituitary cells (14, 15, 23, 24), has been well documented. The inactivating \(I_{\text{CA}}\) is mediated by the low voltage-activated or transient (T)-type Ca\(^{2+}\) channel. Although we observed T-type \(I_{\text{CA}}\) in all three cell types examined, it was more prominent in somatotrophs than in lactotrophs and gonadotrophs. A similar conclusion was reached in a study that directly compared VGCC expression between somatotrophs and lactotrophs. The prominent expression of T-type Ca\(^{2+}\) channels in somatotrophs is reflected by their contribution to the generation of the high amplitude [Ca\(^{2+}\)\(_i\)] transients in spontaneously active somatotrophs (25), whereas its role in other native anterior pituitary cells is not known. The noninactivating \(I_{\text{CA}}\) in pituitary cells is mediated by dihydropyridine-sensitive (L-type) and -insensitive, high-voltage activated Ca\(^{2+}\) channels (10, 21, 24, 26). Unlike the T-type Ca\(^{2+}\) channel, the current voltage-relationship of the sustained \(I_{\text{CA}}\) in all three cell types was similar. However, the sustained \(I_{\text{CA}}\) density was higher in somatotrophs and gonadotrophs than in lactotrophs. Despite the different densities of the sustained \(I_{\text{CA}}\), it is essential to the generation of both spontaneous and agonist-induced AP-driven Ca\(^{2+}\) entry in all native and immortalized pituitary cells (4, 27, 28). In addition, the L-type VGCC has been demonstrated to contribute to the regulation of basal and agonist-stimulated GH and PRL secretion, as well as agonist-induced gonadotropin secretion (28, 29).

In general, the VGCC-dependent rise in [Ca\(^{2+}\)\(_i\)] is sufficient to trigger activation of several Ca\(^{2+}\)-sensitive channels. One such channel is the BK-type K\(^+\) channel, the expression of which has been previously demonstrated in immortalized anterior pituitary cells (30–32), and native intermediate pituitary cells (33). Our studies indicate that BK channels are also expressed in native anterior pituitary cells, and that they are coupled to voltage-gated Ca\(^{2+}\)-influx in all three cell types examined. Moreover, by comparing BK channel activation between somatotrophs, lactotrophs, and gonadotrophs under identical culture and recording conditions, it was demonstrated that BK channel activation was much greater in somatotrophs than in gonadotrophs. Due to the similarities in the voltage-gated \(I_{\text{CA}}\) density and change in [Ca\(^{2+}\)\(_i\)], evoked by the Ca\(^{2+}\)-influx step between the three cell types, the differences in \(I_{\text{BK}}\) activation are most likely due to differences in BK channel expression levels.

In other excitable cells, the colocalization of BK channels with VGCCs facilitates spike repolarization, which limits AP-driven Ca\(^{2+}\) influx. BK channel activation can also influence the frequency of AP-driven [Ca\(^{2+}\)\(_i\)] transients by slowing the pacemaker depolarization (34). In native anterior pituitary cells, the role of BK channels in shaping the frequency and duration of AP-driven Ca\(^{2+}\) entry is not known. Based on our results, we would expect that the relatively high levels of BK channel expression in somatotrophs and lactotrophs would limit AP-driven Ca\(^{2+}\) influx compared with that in gonadotrophs, which exhibit the lowest levels of BK channel expression. However, previous studies have demonstrated that the duration of the AP waveform is longer in somatotrophs and lactotrophs (100–500 msec) than in gonadotrophs (10–100 msec) (10, 35–37). In addition, both the amplitude and duration of the spontaneous, extracellular Ca\(^{2+}\)-dependent [Ca\(^{2+}\)\(_i\)] transients are greater in somatotrophs and lactotrophs than in gonadotrophs (2). It is unlikely that the prolonged duration of AP-driven Ca\(^{2+}\) entry in somatotrophs and lactotrophs is due to the inability of AP firing to activate BK channels, as short Ca\(^{2+}\) influx steps (<25 msec) were sufficient to activate \(I_{\text{BK}}\) in both cell types (Fig. 10). Thus, whether BK channels have an atypical role in regulating the pattern of AP firing and Ca\(^{2+}\) signaling in anterior pituitary cells requires further studies.

All three pituitary cell types also express SK channels (31, 38–40). However, in gonadotrophs SK channels did not appear to be coupled to voltage-gated Ca\(^{2+}\) influx under the conditions used in our study. Similarly, voltage-gated Ca\(^{2+}\) influx activated SK channels in only a small fraction of the somatotrophs and lactotrophs examined. This may be due to the lack of SK channel expression in some somatotroph and lactotroph subpopulations. Consistent with this, activation of Ca\(^{2+}\)-mobilizing TRH receptors leads to activation of SK channels and the concomitant membrane hyperpolarization in GH\(_3\) cells (32), but only a small fraction of lactotrophs exhibited a similar response (41). It is also possible that the SK channels in pituitary cells, as in other cell types (reviewed in Ref. 42), are colocalized with intracellular Ca\(^{2+}\) release sites and can be activated only by Ca\(^{2+}\)-mobilizing receptors, sustained voltage-gated Ca\(^{2+}\) entry, and/or Ca\(^{2+}\)-induced Ca\(^{2+}\) release. For example, in GnRH-secreting neurons, agonist-induced Ca\(^{2+}\) mobilization and the concomitant increase in firing frequency are needed to activate SK channels (43). Similarly, in GH\(_3\) cells, SK channel activation requires high-frequency firing, prolongation of APs by voltage-dependent K\(^+\)
channel inhibitors, or release of Ca\textsuperscript{2+} from intracellular Ca\textsuperscript{2+} stores (31). The lack of a detectable apamin-sensitive I\textsubscript{K} in a majority of the three cell types examined may also be due to its relatively small size compared with the voltage-gated I\textsubscript{K} and the I\textsubscript{SK} in these cells. Other Ca\textsuperscript{2+}-sensitive channels, such as Cl\textsuperscript{-} channels known to be expressed in AtT-20 immortalized cells (8) and native lactotrophs (9) may also be masked.

Several different voltage-gated K\textsuperscript{+} channel subtypes have been identified and characterized in somatotrophs, lactotrophs, and gonadotrophs (44, 45), as well as immortalized pituitary cells (14, 15). One such channel is the transient, 4-AP-sensitive (A-type) K\textsuperscript{+} channel. Direct comparison of the three anterior pituitary cells examined in this study indicates that the expression level of the A-type K\textsuperscript{+} channel is much higher in lactotrophs and gonadotrophs than in somatotrophs. In contrast, these channels have been observed in ovine somatotrophs and may contribute to AP firing and hormone secretion (46). The participation of the A-type K\textsuperscript{+} channel in regulating AP firing in other anterior pituitary cell types is also unclear. In rat lactotrophs, for example, they do not appear to participate in AP generation (37), which may be due to their prominent inactivation at the resting membrane potential in these cells. The participation of the delayed rectifying K\textsuperscript{+} channel during AP firing in anterior pituitary cells is also not clear. In immortalized cells, inhibition of this channel by tetraethylammonium (TEA) increased the duration of the AP (37) and the amplitude of the spontaneous [Ca\textsuperscript{2+}]\textsubscript{i} transients (47), whereas in native rat lactotrophs, TEA did not alter the pattern of AP firing (37). Further studies are required to elucidate the role of both the A-type and delayed rectifying K\textsuperscript{+} channels in the regulation of AP-driven Ca\textsuperscript{2+} signaling in the different anterior pituitary cell types.

Our study was focused on the ionic channels involved in the formation of the AP waveform. Other channels have also been identified in pituitary cells, and they contribute to pacemaking activity in these cells. For example, a TTX-insensitive I\textsubscript{Na} has been observed in somatotrophs (48) and lactotrophs (12) and is critical for maintaining the membrane potential near the threshold for AP firing. In addition, inward rectifier K\textsuperscript{+} channels, including the erg-like K\textsuperscript{+} channel, have been identified in both native (36, 49) and immortalized pituitary cells (50). They contribute to the regulation of both spontaneous and agonist-modulated AP-driven Ca\textsuperscript{2+} entry. The M-type K\textsuperscript{+} channel has also been identified in pituitary cells (41), as well as several ATP-gated P2X receptor channels (51). Preliminary data also suggested the presence of cyclic nucleotide-gated channels in pituitary cells, which may contribute to pacemaking activity (25).

In summary, we examined the cell-type specific expression of the major ionic currents in somatotrophs, lactotrophs, and gonadotrophs that contribute to AP-driven Ca\textsuperscript{2+} entry. All experiments were done under identical experimental conditions on cells from randomly cycling adult female rats. This allowed us to directly compare the expression levels and voltage-dependent properties of several ionic channels between the three anterior pituitary cell types. Our results demonstrate that, although these cells originate from the same precursor cell, they exhibit cell type-specific patterns of ionic channel expression. In particular, the marked differences in BK channel and transient (A-type) K\textsuperscript{+} channel expression between the three cell types are excellent candidates for future investigations into the cell type-specific patterns of spontaneous and receptor-controlled membrane excitability, Ca\textsuperscript{2+} signaling, and hormone secretion.

**MATERIALS AND METHODS**

**Pituitary Cell Culture and Cell Identification**

Anterior pituitary glands were excised from female Sprague Dawley rats (Taconic Farms, Inc., Germantown, NY) and dispersed into single cells using a trypsin/DNase (Sigma, St. Louis, MO) cell dispersion procedure as described previously (51). Enriched somatotroph (20-90%, estimated by immunocytochemistry using specific antibodies provided by the National Hormone and Pituitary Program and Dr. Parlow) and lactotroph (>65%) populations were obtained using a discontinuous Percoll density-gradient cell-separation procedure as described previously (51). Pituitary somatotrophs and lactotrophs were further identified using a combination of cell separation techniques, their distinct morphology, and responses to GHRH and TRH, respectively. Gonadotrophs were initially identified by their distinct morphology and subsequent to experimentation by their unique oscillatory I\textsubscript{K} and/or [Ca\textsuperscript{2+}]\textsubscript{i} response to GnRH, the specific agonist for these cells.

**Electrophysiological Recordings**

Voltage-gated I\textsubscript{Na} and I\textsubscript{Ca} were measured using regular whole-cell recording techniques, whereas voltage-gated I\textsubscript{K} and I\textsubscript{Ca}(A) were measured using perforated-patch recording techniques (52). All 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. A series resistance of < 20 M\textohm was reached 10 min after the formation of a gigahm seal (seal resistance > 5 G\textohm). When necessary, series resistance compensation was optimized and all current recordings were corrected for linear leakage and capacitance using a P/\textminus N procedure. An average membrane capacitance (C\textsubscript{m}) of 4.6 ± 0.2 pF (n = 48), 5.9 ± 0.2 pF (n = 47), and 7.9 ± 0.2 pF (n = 68) was recorded in somatotrophs, lactotrophs, and gonadotrophs, respectively. Pulse generation and data acquisition were done with a PC equipped with a Digidata 1200 A/D interface in conjunction with Clampex 8 (Axon Instruments).

**Simultaneous Measurement of [Ca\textsuperscript{2+}]\textsubscript{i} and I\textsubscript{K}**

To simultaneously monitor [Ca\textsuperscript{2+}]\textsubscript{i} and I\textsubscript{K}, the cells were incubated for 15 min at 37 C in phenol red-free medium 199 containing Hanks’ salts, 20 mM sodium bicarbonate, 20 mM HEPES, and 0.5 mM indo-1 AM (Molecular Probes, Inc., Eugene, OR). The coverslips with cells were then washed twice with modified Krebs-Ringer’s solution containing (in mM): 120 NaCl, 4.7 KCl, 2.0 CaCl\textsubscript{2}, 2 MgCl\textsubscript{2}, 0.7 MgSO\textsubscript{4}, 10
Ionic Channels in Anterior Pituitary Cells

HEPES, 10 glucose (pH adjusted to 7.4 with NaOH) and mounted on the stage of an inverted epifluorescence microscope (Nikon, Melville, NY). A photon counter system (Nikon) was used to simultaneously measure the intensity of light emitted at 405 nm and 480 nm after excitation at 340 nm. Background intensity at each emission wavelength was corrected. Perforated-patch recording techniques (see above) were used to control the membrane potential and monitor $I_m$ in the voltage-clamp recording mode. The data were digitized at 10 kHz using a PC equipped with the Clampex 8 software package in conjunction with a Digidata 1200 A/D converter (Axon Instruments). The $[Ca^{2+}]_i$ was calibrated in vivo as described by Kao (53). Briefly, $R_m$ was determined by exposing the cells to 10 mM Br-A23187 in the presence of Krebs-Ringer’s solution with 2 mM EGTA and 0 mM Ca$^{2+}$ for 0 min; 15 mM Ca$^{2+}$, and 100 mM caffeine was used to determine $R_{max}$. The values used for $R_m$, $R_{max}$, $S_{450}/S_{440}$, and dissociation constant ($K_d$) were 0.677, 2.9, 2.473, and 230 nM, respectively.

**Chemicals and Solutions**

For all $I_x$ recordings, the extracellular medium contained modified Krebs-Ringer salts containing (in mM): 120 NaCl, 4.7 KCl, 1.2 MgCl$_2$, 2.0 CaCl$_2$, 0.7 MgSO$_4$, 10 HEPES, 10 glucose and 1 mM TTX (pH adjusted to 7.4 with NaOH) and the pipette solution contained (in mM): 50 KCl, 90 K-aspartate, 1 MgCl$_2$, and 10 HEPES (pH adjusted to 7.2 with KOH). To isolate the voltage-gated $I_{Na}$ in the extracellular medium contained 200 mM Cd$^{2+}$ and 100 mM TTX. In some experiments, extracellular Ca$^{2+}$ was replaced with equimolar Mg$^{2+}$. To isolate $I_{Ca}$ and $I_{Na}$ in a conventional whole-cell recording configuration were used as previously described (52). The extracellular medium contained Krebs-Ringer’s solution with 20 mM TEA and 2 mM CaCl$_2$ (pH adjusted to 7.4 with NaOH) and the pipette contained (in mM): 120 CsCl, 20 TEA-Cl, 4 MgCl$_2$, 10 EGTA, 9 glucose, 20 HEPES, 0.3 Tris-GTP, 4 Mg-ATP, 14 CrPO$_4$, and 50 U/ml creatine phosphokinase (pH adjusted to 7.2 with Tris base). To isolate INa or ICa, conventional whole-cell recording techniques were used as previously described (52). The extracellular medium contained Krebs-Ringer’s solution with 20 mM TEA and 2 mM CaCl$_2$ (pH adjusted to 7.4 with NaOH) and the pipette contained (in mM): 120 CsCl, 20 TEA-Cl, 4 MgCl$_2$, 10 EGTA, 9 glucose, 20 HEPES, 0.3 Tris-GTP, 4 Mg-ATP, 14 CrPO$_4$, and 50 U/ml creatine phosphokinase (pH adjusted to 7.2 with Tris base). To isolate the $I_{Na}$ or $I_{Ca}$, the intracellular medium contained (in mM): 50 KCl, 90 K-aspartate, 1 MgCl$_2$, 0.7 MgSO$_4$, 10 HEPES, 10 glucose, and 100 mM TTX (pH adjusted to 7.4 with NaOH) and the extracellular solution contained (in mM): 120 NaCl, 2.0 CaCl$_2$, 2 MgCl$_2$, 0.7 MgSO$_4$, 10 HEPES, 10 glucose and 100 mM TTX (pH adjusted to 7.4 with NaOH) and the extracellular solution contained (in mM): 120 NaCl, 2.0 CaCl$_2$, 2 MgCl$_2$, 0.7 MgSO$_4$, 10 HEPES, 10 glucose, and 100 mM TTX.

**Data Analysis**

Data analysis was performed using Clampfit (Axon Instruments). In some cases, the current-voltage relations were fit with a single Boltzmann relation: $\frac{I/I_{max}}{1+e^{-\frac{E_x-E_c}{k}}} = \frac{I/I_{max}}{1+e^{-\frac{E_x-E_c}{k}}}$ where $I_{max}$ is the maximum current, $E_x$ is the membrane potential, $E_c$ is the command potential, and $k$ is the slope factor. The results shown are typical tracing or means ± SEM for at least five recordings. Differences between groups were considered to be significant with $P < 0.05$ or higher, calculated by paired $t$ test or ANOVA, followed by Fisher’s least significant differences test.

**Acknowledgments**


**References**

37. Sankaranarayanan S, Simasko SM 1998 Potassium channel blockers have minimal effect on oscillation of spontaneous action potentials in rat pituitary lactotropes. Neuroendocrinology 68:297–311
47. Charles AC, Piros ET, Evans CJ, Hales TG 1999 L-type Ca$^{2+}$ channels and K$^+$ channels specifically modulate the frequency and amplitude of spontaneous Ca$^{2+}$ oscillations and have different roles in prolactin release in GH3 cells. J Biol Chem 274:7508–7515
54. Van Goor F, Zivadinovic D, Wong AOL, Stojilkovic SS 2000 Calcium-activated, voltage-dependent K$^+$ (BK) channels account for differences in the spiking pattern between spontaneously active rat somatotrophs, lactotrophs, and gonadotrophs. 30th Annual Meeting of the Society for Neuroscience, New Orleans, LA (Abstract 689.9)