Modulation of Ion Channels Underlying Excitation-Secretion Coupling in Identified Lactotrophs and Gonadotrophs

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ABSTRACT

The role of ion channel activity in the response of rat pituitary lactotrophs and gonadotrophs to dopamine (DA) and GnRH, respectively, was investigated. Single lactotrophs and gonadotrophs were unambiguously identified with the reverse hemolytic plaque assay and recordings of membrane potential and current were obtained using whole-cell and single-channel patch-clamp techniques. In lactotrophs, DA inhibited spontaneous electrical activity by activating a K+ conductance that hyperpolarized the cells. A 50 pS K+ channel underlies this response and was activated following agonist binding to a D1 type receptor via a "direct" interaction with a pertussis toxin-sensitive G-protein. In gonadotrophs, GnRH triggers rhythmic hyperpolarizations due to a K+ conductance increase. The K+ channel underlying the GnRH response is an amiloride-sensitive, Ca2+-activated channel. Although both agonists produce hyperpolarizations in their respective target cells via K+ channel activation, differences in intracellular calcium responses probably discriminate the stimulatory (GnRH) and inhibitory (DA) actions on hormone secretion. Each K+ channel type plays a different role in modulating the intracellular Ca2+ levels to yield these actions.

INTRODUCTION

A wide variety of neurotransmitters and peptides that reach the anterior pituitary gland via the hypothalamic-hypophys- nal portal system are known to influence the secretion of hormones. Dopamine (DA) appears to be the primary reg-u-lator of prolactin secretion exerting a tonic inhibitory ac-tion upon lactotrophs [1]. In contrast, LH and FSH secretion from gonadotrophs is stimulated by the action of GnRH [2]. While much is known about the coupling between receptors for these agents and second messenger systems, less is known about the ion channel effectors and electrical events associated with signal transduction in individual cell types. This is, in part, due to the difficulty of identifying specific cell types in a mixed population for individual study by single-cell experimental methods. Recently, our laboratory has overcome this problem by combining the reverse hemolytic plaque assay (RHPA) to identify secretory sub-types [3] with measurements of membrane potentials and ion currents from whole cells and isolated membrane patches [4]. Using these methods, we have explored the actions of DA and GnRH on electrical activity in their respective target cells. Our observations indicate that changes in the activity of ion channels in identified lactotrophs and gonadotrophs induced by these secretagogues can both be fundamental to and occur in parallel with the process of excitation-secretion coupling. The present article reviews and expands upon previously published observations [5-7].

MATERIALS AND METHODS

Cells were dissociated from anterior pituitary glands of either male (gonadotroph) or cycling female (lactotroph) Sprague-Dawley rats using either collagenase (lactotroph) or collagenase and trypsin (gonadotrophs) according to previously published protocols [5, 7, 8]. Lactotrophs and gonadotrophs were identified by the RHPA [3] using antibodies to rat prolactin [6] or bovine LH [7], respectively, and maintained in culture for 1-4 days prior to recording. Gigaseal whole-cell or single-channel techniques [4] were employed to record from RHPA-positive cells. For whole-cell experiments, the external bath contained 150 mM NaCl, 2-5 mM CaCl2, 2.5-5 mM KCl, 1 mM MgCl2, 8-10 mM glucose, 10 mM HEPES buffer (pH 7.4). The intracellular pipette solution contained 120-130 mM potassium aspartate, 20 mM KCl, 0.1 mM guanosine triphosphate (GTP), 2 mM ATP, and 10-20 mM HEPES buffer (pH 7.4). For single-channel measurements in cell-attached patches, the pipette contained 150 mM KCl, 3.1 mM MgCl2, and 15 mM HEPES buffer.

Secretagogues and other agents were applied directly to the solution bathing the cells or by using a U-tube [9]. All experiments were performed at room temperature (20-25°C).

RESULTS

DA Alters Spontaneous Electrical Activity of Lactotrophs Via an Increase in K+ Conductance

During whole-cell recording, RHPA-identified lactotrophs often exhibited spontaneous electrical activity that varied from periodic calcium-dependent action potentials to random fluctuations in membrane potential around a mean level of -25 to -35 mV (Fig. 1, A and C). Brief applications of DA hyperpolarized these cells and halted cal-
DA Opens K Channels Via D<sub>2</sub>-Type Receptors Coupled to Guanine Nucleotide Binding Proteins, But Independent of Soluble Second Messengers

The actions of DA on membrane potential, macroscopic whole-cell currents, and single-channel currents were mimicked by the D<sub>2</sub> receptor-selective agonists RU24213 and quinpirole [6, 7]. In addition, these effects were blocked by preincubation of the cells with the D<sub>2</sub> receptor antagonists, sulpiride and (+)butaclamol [5]. This indicates that activation of D<sub>2</sub> class DA receptors underlies the electrical changes induced by DA.

The actions of DA were also blocked by either preincubation with pertussis toxin (PTX) or acute intracellular dialysis with GDPBS contained in the patch electrode [6], indicating that a guanine nucleotide binding protein (G-protein) is involved in the signal transduction pathway. Dialysis of GTPyS into the cell from the patch electrode mimicked the action of DA (Fig. 3), bypassing the D<sub>2</sub> receptor and directly activating the relevant G-protein(s).
FIG. 2. Macroscopic and single-channel ion currents induced by DA (100 nM) in lactotrophs. A) Outward current response to DA (bar) in a cell voltage clamped to -40 mV. B) Current-voltage relations for the DA-sensitive current (computer subtraction of currents without DA from those recorded in the presence of DA) in the presence of either 5 mM (filled circles) or 50 mM (open circles) extracellular K⁺. Both external and internal Ca²⁺ was buffered to low levels with EGTA. C) Representative traces of ion channel activity in 2 cell-attached membrane patches in the absence (left) or presence (right) of DA in the patch electrode. Patch electrode was held at +40 mV and contained 50 mM K⁺. D) Composite current-voltage relationship for DA-activated single K channels from 8 cell-attached membrane patches (mean ± SD). Solid line is a least-squares regression fit to the data points yielding a single-channel conductance of 49.5 pS.

FIG. 3. Intracellular GTPyS mimics the response produced by DA. Traces represent membrane potential recordings from two cells on the same coverslip. In one case, the cell was dialyzed by GTP (100 μM) in the patch electrode and exhibited a stable membrane potential that responded to the application of 100 nM DA (bar). The other cell was dialyzed with 50 μM GTPyS and spontaneously hyperpolarized after achieving the whole-cell recording configuration (time = 0 min). Subsequent application of DA produced only a small additional change of potential.
In contrast to lactotrophs, RHPA-positive gonadotrophs exhibited little spontaneous electrical activity in culture. Resting potentials of -30 to -45 mV were normally observed under unstimulated conditions [8]. When GnRH was applied to individual gonadotrophs, they underwent strong rhythmic hyperpolarizations, each lasting 1-3 sec and often terminated by several action potentials (Fig. 4A). When cells were voltage-clamped at -50 mV, GnRH induced rhythmic oscillations of outward current with a periodicity similar to that seen for the hyperpolarizations (Fig. 4B). The oscillatory outward currents diminished over several minutes following removal of GnRH.

**GnRH Triggers Oscillations in Membrane Potential and Current in Gonadotrophs**

In contrast to lactotrophs, RHPA-positive gonadotrophs exhibited few spontaneous electrical activity in culture. Resting potentials of -30 to -45 mV were normally observed under unstimulated conditions [8]. When GnRH was applied to individual gonadotrophs, they underwent strong rhythmic hyperpolarizations, each lasting 1-3 sec and often terminated by several action potentials (Fig. 4A). When cells were voltage-clamped at -50 mV, GnRH induced rhythmic oscillations of outward current with a periodicity similar to that seen for the hyperpolarizations (Fig. 4B). The oscillatory outward currents diminished over several minutes following removal of GnRH.

**DA Does Not Modulate the Activity of Other Channels in Lactotrophs**

It has been reported that DA can increase the activity of voltage-gated K channels [11] and decrease voltage-gated Ca\(^{2+}\) currents [12] in lactotrophs from lactating female rats. In recent experiments on RHPA-identified cells from normal cycling female rats using both whole-cell and perforated patch [13] recording techniques, we have consistently failed to observe such changes (data not shown). This suggests that the K\(^+\) channel described above is the primary, if not exclusive, effector target for D\(_2\)-receptor-mediated changes in excitability.

**GnRH-Induced Current Oscillations Reflect Opening of Ca\(^{2+}\)-Activated K Channels**

The oscillatory currents induced by GnRH reversed direction from outward to inward when the membrane potential was clamped more negative than E\(_K\). Furthermore, the voltage at which this reversal occurred shifted with changes in the extracellular K\(^+\) concentration in accord with the predicted changes in E\(_K\), indicating that the currents arose from activation of a K\(^+\)-selective ion channel [7].
During whole-cell recordings where the patch pipette contained an intracellular Ca\textsuperscript{2+} buffering solution (free Ca\textsuperscript{2+} estimated to be 120 nM), GnRH application failed to induce normal oscillatory currents, and only a small, slow, outward current was seen (Fig. 5A). This small outward current was reduced by the application of 100 nM apamin, a selective blocker of small conductance Ca\textsuperscript{2+}-activated K channels (SK channels)\cite{14}. Current oscillations induced in the absence of exogenous calcium buffers were completely eliminated by exposure to apamin (Fig. 5B). In contrast, the application of 5 mM tetraethylammonium (TEA\textsuperscript{+}), a potent blocker of large conductance Ca\textsuperscript{2+}-activated K channels and a general blocker of other K channel types, only partially diminished the oscillatory response to GnRH (Fig. 5B). These results indicate that the outward currents arose primarily, if not exclusively, from activation of SK-type Ca\textsuperscript{2+}-activated K channels.

**Current Oscillations Arise from Cyclic Release of Ca\textsuperscript{2+} from Intracellular Stores Mediated by Inositol Trisphosphate (IP\textsubscript{3})**

It is known from studies using fluorometric Ca\textsuperscript{2+} indicators that GnRH induces cyclic increases in intracellular Ca\textsuperscript{2+} in suspensions of gonadotrophs or in single cells\cite{15}. These oscillatory Ca\textsuperscript{2+} changes are temporally correlated with the hyperpolarizing events and outward currents\cite{16; Tse, Tse, Almers, and Hille, unpublished observations}, suggesting a causal relationship between Ca\textsuperscript{2+} elevation and outward current. It is therefore of interest to explore the nature of the sources giving rise to the Ca\textsuperscript{2+} elevations and the signal transduction mechanisms involved.

GnRH stimulates phosphoinositide turnover in gonadotrophs through activation of a PTX-insensitive G-protein\cite{17}. Accordingly, introduction of the active D-isomer of IP\textsubscript{3} into single gonadotrophs via dialysis from the patch electrode triggers current oscillations in the absence of GnRH stimulation\cite{7}. Furthermore, dialysis with heparin, a competitive antagonist of IP\textsubscript{3}, blocks the electrophysiological effects of 1 nM GnRH\cite{7}. The involvement of a G-protein in the signal transduction pathway is indicated by the observation that 100 µM GTP\textsubscript{y}S in the electrode renders the response to brief applications of GnRH irreversible\cite{7} (Fig. 6). The GnRH response can also be prevented by including 2 mM GDP\textsubscript{y}S in the electrode\cite{7}. 
When single gonadotrophs were bathed in a solution containing 6 mM Mg^{2+}, 1 mM EGTA, and no calcium, GnRH elicited current oscillations that persisted for several minutes in the absence of extracellular Ca^{2+} in the majority of cells examined (Fig. 6). In addition, intracellular IP_{3} triggered similar oscillations in the absence of extracellular Ca^{2+}. Taken together these data suggest that the calcium oscillations seen in gonadotrophs arose from cyclic IP_{3}-mediated release of internal Ca^{2+}.

**DISCUSSION**

It is clear that both rat lactotrophs and gonadotrophs exhibit characteristic changes in electrical activity when challenged with agents widely accepted as the principle mediators by which the central nervous system controls secretion of prolactin, FSH, and LH. There are similarities and differences in the nature of the responses to either DA or GnRH that point to the common involvement of K'-selective ion channels, yet also reveal a diversity of specific mechanisms underlying the control of hormone secretion.

In their respective target cells, both secretogogues (a) induce dramatic hyperpolarizations of the membrane potential, (b) increase membrane K' permeability by activating specific classes of K' channels, (c) couple to effector K' channels via G-proteins linked to their respective membrane receptors, and (d) persist in the absence of extracellular Ca^{2+}. Clear differences exist between DA and GnRH responses, however, in terms of the type of K' channels activated and the signal transduction processes involved. The K' channels activated in lactotrophs by DA exhibit mild inward rectification, are insensitive to apamin (Oxford, unpublished observation) and TEA' [6], and can be robustly activated at low intracellular Ca^{2+} levels (< 100 nM). In contrast, the K' channels activated by GnRH in gonadotrophs readily conduct outward K' fluxes, are highly sensitive to apamin, and are critically dependent upon elevations in intracellular Ca^{2+}. In the case of DA-activated K' channels, a PTX-sensitive G-protein appears to couple "directly" to channel opening without an intermediate second messenger [18]. On the other hand, GnRH appears to couple to a signal transduction cascade involving a PTX-insensitive G-protein (e.g., G_{o} [19]) that activates phospholipase C, elevating IP_{3}, which in turn releases Ca^{2+} from an internal store(s), triggering the opening of apamin-sensitive SK channels.

Given the similarity in the signature hyperpolarizing responses of these two pituitary cell types to their agonists, how then is it possible that the underlying K channel events contribute to the inhibition of prolactin secretion in lactotrophs, yet be involved in the stimulation of LH secretion in gonadotrophs? We speculate that the answer to this apparent paradox lies in the consequences of the response of each ion channel to intracellular Ca^{2+} levels in the two cell types. Activation of K' channels by DA in lactotrophs is not only independent of intracellular Ca^{2+}, it is not accompanied by changes in phosphoinositide metabolism [20], and actually results in a characteristic decrease in cytoplasmic Ca^{2+} [21; J. Rendt, unpublished observations]. This reduction in intracellular Ca^{2+} most likely arises from the inhibition of spontaneous Ca^{2+}-dependent action-potential activity that attends the hyperpolarization. Given that the hyperpolarizing response to DA is very resistant to desensitization (e.g., Fig. 1C and Oxford, unpublished observations), it is well suited to support a chronic suppression of prolactin secretion during normal periods of dopaminergic influence of the pituitary by the hypothalamus [22].

In the case of the gonadotrophs it is likely that the GnRH-triggered changes in phosphoinositide metabolism and the resultant cyclic release of Ca^{2+} from intracellular stores supports the release of LH and FSH. Preliminary measurements of secretion via cell membrane capacitance changes suggest, in fact, that Ca^{2+} oscillations, current oscillations, and vesicular release are tightly temporally coupled [Tse, Tse, Almers, and Hille, unpublished observations]. Since exocytosis can be triggered by GnRH when SK channels are inhibited, it is unlikely that the opening of these channels plays an essential role in excitation-secretion coupling. Similar conclusions have been reached for the opening of Ca^{2+}-activated K channels in pituitary tumor cells during thyrotropin-releasing hormone stimulation of prolactin secretion [23]. As noted above, RHPA-identified gonadotrophs do not normally exhibit action-potential activity in the unstimulated state; however, calcium-dependent spikes are observed following each cyclic hyperpolarization when stimulated by GnRH. These "anode break" action potentials most likely arise from the reactivation of voltage-dependent Na and Ca channels by the strong hyperpolarization. Voltage-dependent calcium currents have been observed in gonadotrophs and are half inactivated at normal unstimulated resting potentials (e.g., -45 mV) [Tse and Hille, unpublished results]. Activation of these "re-primed" calcium channels as the membrane potential returns from the cyclic hyperpolarization would provide a pathway for the influx of extracellular Ca^{2+} to both directly promote secretion and replenish intracellular stores aiding in the maintenance of the oscillatory response. Thus, the opening of SK channels and subsequent hyperpolarization of gonadotrophs may serve an indirect role to reprime a potent influx pathway for calcium to maintain internal Ca^{2+} stores that play a more direct role in the excitation-secretion coupling process.

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