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Apamin-sensitive potassium channels mediate agonist-induced oscillations of membrane potential in pituitary gonadotrophs

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In cultured rat pituitary gonadotrophs, gonadotropin-releasing hormone (GnRH) induces rapid hyperpolarization of the cell membrane and causes cessation of the spontaneous electrical activity present in non-stimulated cells. This initial response to GnRH is followed by slow oscillations of membrane potential (V_m) which often exhibit brief bursts of action potentials (AP) fired from the peak of the oscillations. The hyperpolarization waves are synchronous with GnRH-induced elevations of cytoplasmic Ca^{2+} concentration ($[Ca_2]_i$), such that V_m maxima alternate with the peak values of $[Ca^{2+}]_i$. The V_m oscillations result from repetitive activation of apamin-sensitive K^+ channels by cytoplasmic Ca^{2+}. Thus, GnRH activation of Ca^{2+} mobilization can generate a bursting pattern of membrane potential through the activation of K^+ channels against a background of spontaneous electrical activity.

Pituitary; Intracellular Ca^{2+}; Ca^{2+} channel; Ca^{2+}-sensitive K^+ channel

1. INTRODUCTION

The stimulatory action of GnRH on secretion in cultured pituitary gonadotrophs is mediated by activation of phospholipase C and hydrolysis of polyphosphoinositides, with rapid production of Ins(1,4,5)P_3 and diacylglycerol [1]. The ensuing GnRH-induced rise in $[Ca^{2+}]_i$ occurs in an oscillatory manner [2–4] with dose-dependent frequency between 0.05 and 0.3 Hz, or in a non-oscillatory manner at high agonist concentrations [5]. The initiation of Ca^{2+} oscillations is independent of Ca^{2+} entry, but the continuation of the response requires dihydropyridine-sensitive Ca^{2+} influx [6]. This observation suggests that voltage-sensitive Ca^{2+} channels (VSCC), and thus the electrical activity of the plasma membrane, are also involved in the response to GnRH. Although the electrophysiology of the gonadotrophs is not well characterized, reports on the expression of L- and T-types Ca^{2+} channels [7] and the occurrence of action potential-like events [8] in rat gonadotrophs further support the involvement of electrical activity in GnRH action.

In order to understand how plasma membrane potential, and thus voltage-sensitive elements, participate in the physiology of gonadotrophs, we measured V_m in isolated gonadotrophs using the perforated patch clamp technique [9]. The data presented here demonstrate that GnRH action is associated with a marked change in the pattern of spontaneous electrical activity of gonadotrophs; GnRH-induced Ca^{2+} mobilization evokes a complex oscillatory pattern of V_m due to the activation by Ca^{2+} of apamin-sensitive K^+ channels, which in turn causes periodic hyperpolarization of the plasma membrane.

Abbreviations: GnRH, gonadotropin-releasing hormone; VSCC, voltage-sensitive Ca^{2+} channels; V_m, membrane potential; AP, action potentials.

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2. MATERIALS AND METHODS

2.1. *Cell culture*

Gonadotrophs were obtained from 2-week ovariectomized adult female rats. Pituitary glands were enzymatically dispersed and a cell population enriched in gonadotrophs was prepared by elutriation as described previously [7]. Cells were cultured in Hanks' Medium 199 with 10% horse serum, for 24–72 h at 37°C (95% air, 5% CO_2).

2.2. *Membrane potential and whole cell current recordings*

Culture medium was replaced by a solution containing (in mM): 125 NaCl, 4 KCl, 2.5 $NaHCO_3$, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES (pH adjusted to 7.35 with NaOH), and 10 glucose. Cells were placed on the stage of an inverted microscope and membrane potential was measured under current clamp conditions using the nystatin-perforated patch technique. For this purpose, microelectrodes (3–6 MΩ tip resistance) made from soft glass were filled with a high K^+ solution (in mM: 75 K_2SO_4, 10 KCl, 1 $MgCl_2$, 10 HEPES; pH adjusted to 7.2 with NaOH). The pipette was backfilled with the same solution, to which nystatin (Sigma) dissolved in dimethylsulfoxide was added to a final concentration of 100–150 μg/ml. Membrane potential and whole cell membrane current measurements were carried out using an EPC-7 patch amplifier (List). Liquid junction potentials were compensated before starting each experiment. The current signal was low

pass filtered at 1 kHz using a Bessel filter (Frequency Devices). Permanent records of both membrane potential and membrane current were made on analogue magnetic tape (Racal Recorders). All experiments were carried out at 20–24°C.

2.3. *Cytoplasmic Ca^{2+} measurements*

$[Ca^{2+}]_i$ concentration was measured by microfluorometry using Indo-1 as previously described [5]. Cells plated on glass coverslips were loaded with the indicator by incubation in modified Krebs' solution containing Indo-1 ester (2 μM) at 37°C for 1 h. Cells were placed on the stage of a Nikon inverted microscope (epifluorescence mode) and the fluorescence at 410 and 480 nM was measured using two photomultipliers. The output from the photomultipliers was used to calculate the ratio F_{410}/F_{480} using an analog divider; this device allowed the continuous monitoring and recording of the Ca^{2+} signal.

3. RESULTS AND DISCUSSION

Rat gonadotrophs in culture often exhibited spontaneous electrical activity (58%, n = 125 cells) with a frequency of 1.5 Hz to 0.1 Hz (Fig. 1A and B). These action potentials (AP) were insensitive to tetrodotoxin (15 μM), but were suppressed in the absence of extracellular Ca^{2+} or in the presence of nifedipine (2–5 μM, not shown). Membrane depolarization by current injection increased the frequency of AP or induced electrical activity in silent cells.

Low GnRH concentrations (0.01–1 nM) caused a transitory cessation of such spontaneous AP, followed by a complex pattern of V_{max} oscillations with a frequency of 0.1–0.3 Hz. Each cycle consisted of a phase of rapid hyperpolarization to a level of −65 to −80 mV (−71.0 ± 6.6 mV, n=10) and a slow depolarization that often led to the firing of AP (1–6 spikes in 47 of the 95 cells exposed to the agonist) (Fig. 1A). The remaining cells showed oscillations without spikes, even though many of them fired spontaneous AP in the non-stimulated state. Higher GnRH concentrations (10–100 nM) induced a biphasic change in V_m with a rapid and sustained hyperpolarization to −69.4 ± 3.9 mV (n=5) followed by oscillations, as shown in Fig. 1B. In electrically silent cells, GnRH caused immediate hyperpolarization of the membrane and elicited oscillations comparable to those of active cells (Fig. 1C). In occasional spontaneously hyperpolarized cells (V_m −94 and −96 mV) GnRH had a depolarizing effect, bringing the V_m to ca. −72 mV and generating inverted oscillations between this level and the original more negative value (Fig. 1D). Among the population of cells from which records were obtained (130), more than 95% responded to GnRH with hyperpolarization and/or oscillations of V_m.

Since GnRH is known to evoke $[Ca^{2+}]_i$ oscillations

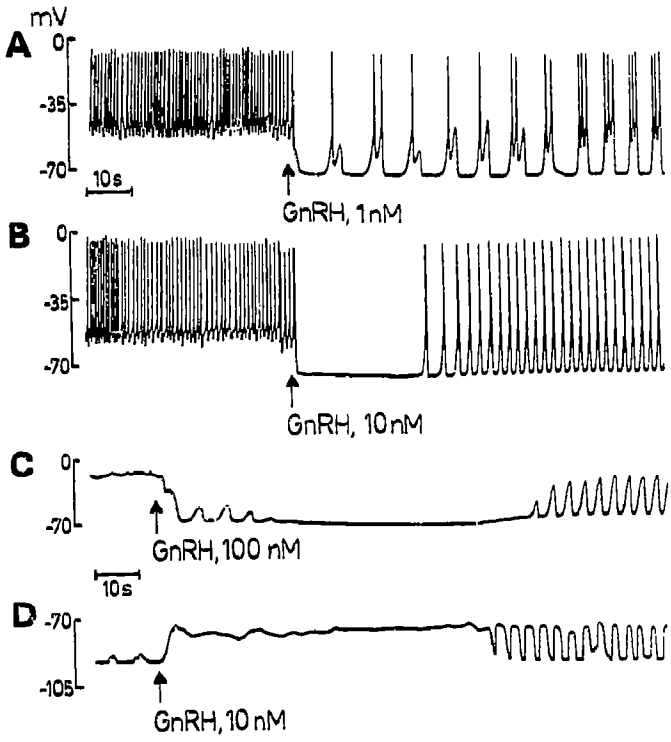

Fig. 1. (A–D) Membrane potential records from 4 independent gonadotrophs. 100 μl aliquots of GnRH solutions in extracellular medium were added at the times indicated by arrows to obtain the final concentrations given below each trace.

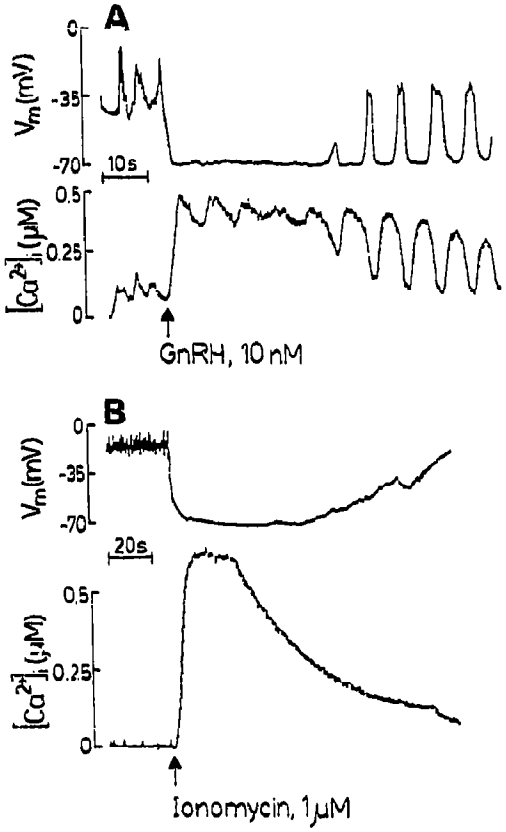

Fig. 2. (A) Simultaneous records of membrane potential (upper trace) and Ca^{2+} (lower trace) obtained from a single gonadotroph. The arrow indicates the time of addition of GnRH. (B) Effect of ionomycin on membrane potential (upper trace) and Ca^{2+} (lower trace) in gonadotrophs.

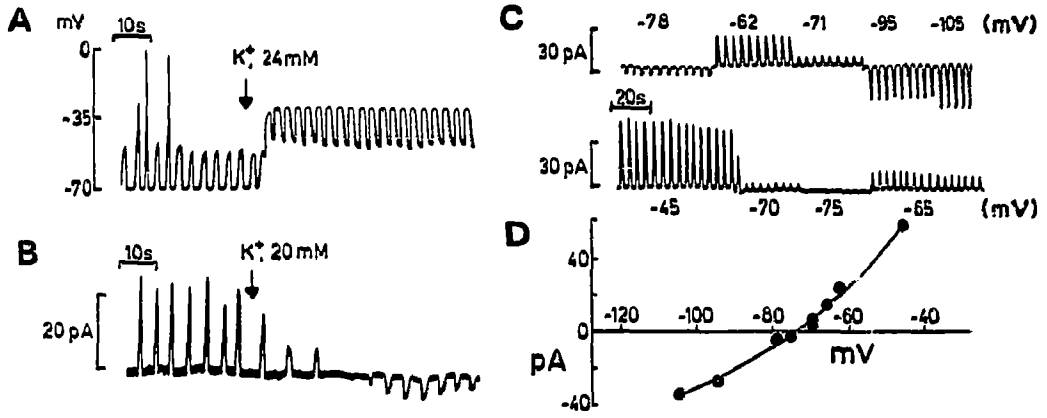

Fig. 3. (A) Effect of increasing $[K^+]_o$ from 4 to 24 mM on membrane potential of a gonadotroph stimulated with 1 nM GnRH. (B) Changes in extracellular K^+ concentration shift the reversal potential for the oscillatory current recorded from a gonadotroph stimulated with 1 nM GnRH; V_m was maintained at −50 mV. (C,D) Current–voltage relationship for the oscillatory current recorded from a GnRH-stimulated gonadotroph. Upward deflections of the records represent outward current. Membrane potentials (in mV) are indicated above and below the traces.

with a frequency in the same range of V_m oscillations [5]. It appeared likely that the two oscillators could be coupled. Simultaneous measurement of both parameters in single gonadotrophs showed that the initial rise in $[Ca^{2+}]_i$ was paralleled by hyperpolarization to the level seen in independent recordings (Fig. 2A). The subsequent oscillations of $[Ca^{2+}]_i$ were in phase with oscillations in V_m; higher $[Ca^{2+}]_i$ corresponded to hyperpolarized phases of oscillations. The generation of a non-receptor mediated increase of $[Ca^{2+}]_i$ by addition of a Ca^{2+} ionophore, ionomycin (0.5–2 μM), to the extracellular solution mimicked the initial effect of GnRH and hyperpolarized the V_m to −67 ± 8.1 mV (n=3, Fig. 2B).

Elevation of extracellular K^+ concentration to 12, 24 (Fig. 3A) or 34 mM resulted in displacement of the hyperpolarized phase of oscillations to an extent close to that predicted for the change in the equilibrium potential for K^+; changes in Cl^- concentration had negligible effects on V_m (not shown). The current involved in the oscillations of V_m was directly measured by voltage-clamping the V_m in 21 cells stimulated with GnRH. At all command potentials tested (−120–0 mV) an oscillatory current was observed, which reversed its direction at −71 ± 2.5 mV (outward direction at potentials positive to this level, Fig. 3C,D). Changes in extracellular K^+ concentration shifted the reversal potential of the oscillatory current, which corresponds to the results obtained in clamp conditions (Fig. 3B). These combined results indicate the existence of a Ca^{2+}-activated K^+ current commanding the oscillatory electrical activity of GnRH-stimulated gonadotrophs.

Neither 10 mM tetraethylammonium nor 100 nM charybdotoxin had any effect on V_m or the oscillatory currents recorded from GnRH-stimulated gonadotrophs (not shown). 1 μM apamin, a selective blocker of small conductance Ca^{2+}-activated K^+ channels [10], markedly altered the pattern of oscillations of V_m and shifted the hyperpolarized level to ca. −38 mV (n=4). Thus, apamin diminished the amplitude of oscillations (n=4; Fig. 4A) or completely abolished them (n=3, not shown). Stimulation of cells with GnRH in the presence of apamin was not followed by the hyperpolarized phase observed in the absence of the toxin; however, GnRH modified the pattern of electrical activity and induced small amplitude oscillations of V_m in the range close to −30 mV (n=5; Fig. 4B). When added to GnRH-stimulated cells under voltage clamp, 1 μM apamin

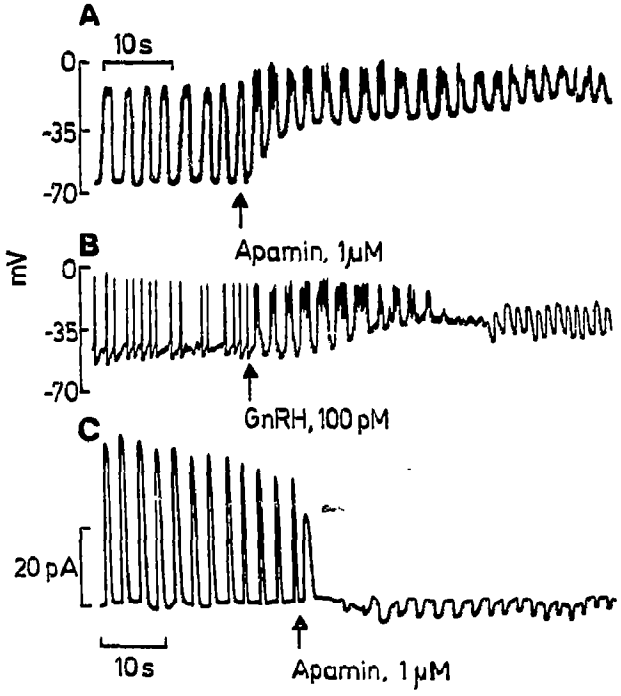

Fig. 4. (A) Effect of apamin on membrane potential oscillations stimulated by GnRH. (B) membrane potential response to GnRH in the presence of 1 μM apamin in the extracellular solution. (C) Effect of apamin (1 μM) on the oscillatory current induced by GnRH in a gonadotroph with membrane potential controlled at −50 mV.

completely blocked the oscillatory K^+ current in 4 out of 10 cells and in the remainder abolished the K^+ component and left a much smaller oscillatory current with a V_m of ca. −36 mV (Fig. 4C).

Taken together, our results indicate that the activation by Ca^{2+} of K^+ channels is the mechanism responsible for the generation of V_m oscillations and the associated firing of bursts of AP in gonadotrophs. This pattern partially fits early models proposed to explain the oscillatory electrical activity of pancreatic β cells [11,12]. However, the maintainance of V_m oscillations in cells spontaneously hyperpolarized or without firing of AP and oscillations of the Ca^{2+}-activated current under voltage clamp conditions supports the view that entry through VSCC is not the immediate source of Ca^{2+} for the occurrence of oscillations, as proposed in such models. Since the response to GnRH in gonadotrophs is mediated by activation of phospholipase C and the production of Ins(1,4,5)P_3, and thapsigargin, a selective blocker of the endoplasmic reticulum Ca^{2+}-ATPase, abolishes $[Ca^{2+}]_i$ and K^+ current oscillations [13], it is probable that the activity of a cytoplasmic oscillator, rather than a process intrinsic to the plasma membrane, commands the oscillatory electrical activity in gonadotrophs. In a number of electrically silent cells, ranging from *Xenopus leavis* oocytes [14] to pancreatic acinar cells [15], Ins(1,4,5)P_3-mediated changes in $[Ca^{2+}]_i$ affect V_m through the activation of Ca^{2+}-dependent channels, but without generation of AP. The novel combination of spontaneous electrical activity, expression of a Ca^{2+}-activated K^+ current, and Ins(1,4,5)P_3-mediated oscillations of $[Ca^{2+}]_i$ enables the gonadotroph to generate a characteristic and complex pattern of V_m oscillations and 'bursting'. A similar mechanism has been proposed to operate in pancreatic β cells [16].

Apamin-sensitive K^+ channels, which are involved in the afterhyperpolarization of neurones and skeletal muscle [17,18], are also expressed in pituitary cell lines [19] and provide the link between $[Ca^{2+}]_i$ and V_m in gonadotrophs. In GH_3 cells this class of channels is transiently activated, mediating the hyperpolarization of the membrane in response to TRH, before the recovery of AP firing [19]. In gonadotrophs, the activation of these channels continues throughout the response to GnRH; the physiological importance of the apamin-sensitive channel-mediated periodic hyperpolarization remains to be elucidated. It is reasonable to postulate that it is associated with the control of Ca^{2+} signalling in gonadotrophs; V_m oscillations may protect the system from the inactivation of voltage-gated Ca^{2+} channels, a phenomenon observed in TRH-stimulated GH_3 cells [20] as well in gonadotrophs stimulated with high doses of GnRH [21]. The operation of these channels, if not important in the early phase of the response, is crucial for the sustained $[Ca^{2+}]_i$ response to GnRH.

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