Electrical activity in endocrine pituitary cells in situ: A support for a multiple-function coding

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Abstract The anterior pituitary is an endocrine gland that controls basic body functions. Pituitary cell functioning depends on membrane excitability, which induces cytosolic calcium rises. Here, we reported the first identification of small-amplitude voltage fluctuations that controlled spike firing in endocrine cells recorded in situ. Three patterns of voltage fluctuations were distinguishable by their durations (1–100 s). These patterns could be ordered on top of each other, namely in response to secretagogues. Thus, pituitary endocrine cells express in situ a cell code in which small-amplitude voltage fluctuations lead to a multimodal arrangement of spike firing, which may finely tune calcium-dependent functions.

Key words: Timing information; Action potential; Calcium signaling; Exocytosis; Gene expression; Growth

1. Introduction

In the anterior pituitary, electrical activity in endocrine cells was reported more than 20 years ago [1]. Electrical activity has received much attention as a potential mechanism that helps encode numerous functions in endocrine pituitary cells. This is based on the fact that each spike causes a transient rise in cytosolic calcium [2], one of the most versatile second messengers [3]. This prompted several investigators to study the mechanisms underlying electrical activity, mostly in cells isolated from their tissue context (clonal cell lines [GH_3, GH_4C_1, GH_3/B_6, AtT20...], cultured human adenoma cells and primary cultures of pituitary cells from various species [rat, bovine, sheep...]). Most of these studies focused on the families of voltage-gated ion channels (Na^+ channels and T-type Ca^{2+} channels) that displayed an increase in open probability at thresholds just above resting membrane potentials [4]. This led to the current consensus that spike firing was modulated mostly by the switching on/off of slowly activating conductances (inward K^+ conductance, Ca^{2+}-sensitive conductances, tetrodotoxin-insensitive Na^+ conductance...) that steadily depolarized or hyperpolarized the membrane potential in response to secretagogues [5–10].

However, other findings of potential interest were observed in isolated cells. For instance, basal membrane potential fluctuations have been reported in primary-culture dispersed pituitary cells [4,11–13] and clonal pituitary cells [14–17]. Although these events were not often quantified and examined in detail, their activities were occasionally shown to be associated with action potential firing in basal conditions [4] or upon agonist stimulation [16]. Here we investigated whether these voltage fluctuations would play a significant role in action potential firing in endocrine pituitary cells when they are recorded in situ. To do so, we applied the acute slice tissue preparation – commonly used in brain studies – to the pituitary parenchyma [18–20]. Perforated patch-clamp conditions were chosen to minimize dialysis artifacts during long-lasting electrical recordings [4,6,7]. We discovered that most pituitary cells displayed small-amplitude voltage fluctuations in acute pituitary slices. Moreover, we identified a multimodal clustering of these membrane potential deflections that provided a subtle coding mode of action potentials in situ.

2. Materials and methods

2.1. Preparation of pituitary slices

The procedure was mainly as previously described [18–20]. Briefly, the pituitary gland was dissected from young (6–10 weeks) male animals and cooled to 2–4°C in gassed (5% CO_2–95% O_2) Ringer's saline containing (in mM): 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 12 glucose, and buffered to pH 7.4. The gland was then either glued onto an agarose cube (for both guinea pig [18] and rat [19] pituitaries) or immobilized within a droplet of ultra-low temperature gelling agarose (mouse pituitaries) [20], and transferred on the stage of a vibratome (Leica VT 1000S, Leica, Nussloch, Germany). Coronal slices (150–250 μm thickness) were generated and transferred in a humidified incubator until used for electrophysiological recording. Only cells from the anterior pituitary part were recorded.

2.2. Electrophysiology

For recording, slices were transferred to a chamber attached to the stage of an upright microscope (Axioskop FS, Zeiss, Le Pecq, France) continuously superfused with Ringer's saline at 32°C. Cells were viewed with a 63×0.9 numerical aperture water immersion objective lens. Membrane potential was recorded in the perforated whole-cell patch-clamp technique. Patch pipets were pulled to a resistance of 4–8 MΩ from borosilicate glass (1.5 mm outer diameter, 1.17 mm inner diameter) and filled with the following internal solution (in mM): 140 potassium-gluconate, 2 $MgCl_2$, 1.1 EGTA, 5 HEPES, and 100 μg/ml nystatin or amphothericin B [6,7] (pH 7.2). Membrane perforation with antibiotics was usually achieved within 20 min after seal formation. Cells with an access resistance over 50 MΩ were discarded.

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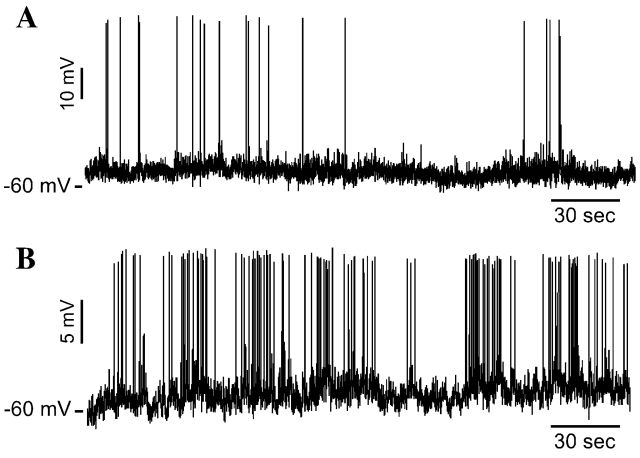

Fig. 1. In situ electrical activity in pituitary endocrine cells. Cells were recorded with the perforated patch-clamp technique, which kept the intracellular milieu intact in guinea-pig anterior pituitary slices. A: Spontaneous all-or-none action potentials occurred randomly in one recorded cell. B: In another spontaneously active cell, action potentials were often grouped as clusters of spikes firing at high frequencies (called 'bursts'). Note the presence of small-amplitude voltage fluctuations, namely during burst epochs.

Membrane potential was recorded under current-clamp conditions using a List EPC-9 patch-clamp amplifier (Heka Elektronik, Lambrecht/Pfalz, Germany) and filtered at 3 kHz. Patch-clamp signals were acquired on a digital recorder DAT (DTR-1800, Biologic, France) and analyzed using Pulse+Pulse Fit software (version 8.3, Heka Electronik). Acquired data were processed for analysis with Igor Pro 3.16 software (Wavemetrics, Lake Oswego, OR, USA). Briefly, both amplitude and duration of all voltage deflections with values above a threshold (noise of current-clamp recordings) were measured along membrane potential recordings. Data were fitted with Gaussian distributions using the curve fitting tool of Igor software. GRF was purchased from Bachem (Voisins-le-Bretonneux, France). Numerical data are expressed as the mean ± S.E.M. Data illustrated in Figs. 1, 3, 4 and 5 were obtained in guinea-pig pituitary slices.

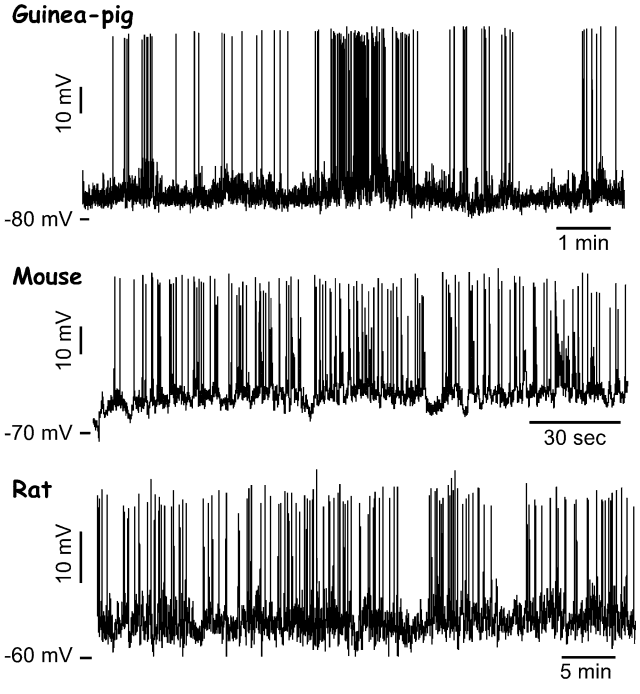

Fig. 2. In situ electrical activity in pituitary cells issued from different mammalian species. Endocrine cells were electrically recorded in acute pituitary slices. Top: guinea-pig. Middle: mouse. Bottom: rat. Recordings were made at zero current level.

3. Results

Individual endocrine pituitary cells were randomly chosen at the surface of the slice for perforated patch-clamping. After perforating the membrane patch in cells from guinea-pig pituitary slices, the resting membrane potential was -60.8 ± 2.9 mV ($n = 32$). Spontaneous all-or-none action potentials were recorded in 69% of patched cells. In 32% of these active cells, action potentials fired randomly (Fig. 1A). In the remainder, groups of spikes firing at a relatively high frequency (around 1 Hz) were detectable (Fig. 1B). Similar profiles of spontaneous electrical activity were observed in both mouse and rat preparations (Fig. 2).

Small-amplitude deflections of the membrane potential were observed in most cells (Figs. 1 and 2). They displayed a broad range in both amplitude and duration. Therefore, we analyzed both parameters with a software for voltage detection over long periods of recording time (30–1800 s). Three modal distributions of deflection durations were detected (Fig. 3A). They were centered around mean values of 0.391, 9.409 and 61.159 s, respectively. Similarly, three distributions of deflection amplitude were also detected (Fig. 3B). These three populations were significantly different (one-way analysis of variance, $P < 0.0001$) with average values that rose as fluctuation durations increased (Fig. 3). Close inspection of these depolarizing events revealed their relationship with the triggering

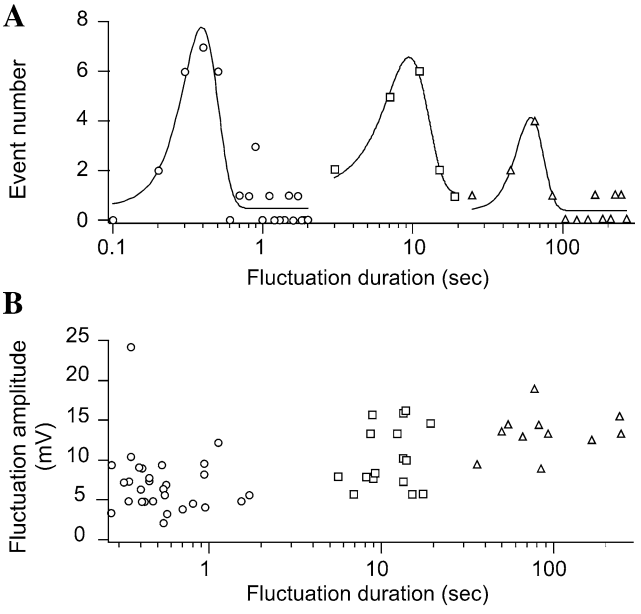

Fig. 3. Duration and amplitude of voltage deflections. A: Number of voltage deflections as a function of deflection durations. Three Gaussian distributions could be fitted around peak levels of 0.3, 9.4 and 61.1 s, respectively. B: Amplitude of voltage deflections as a function of deflection durations. Amplitude values that corresponded to the three distributions of deflection durations could also be classified as three distinct data sets according to their mean values ($V_1 = 6.8 \pm 0.7$ mV [circles], $V_2 = 10.4 \pm 1$ mV [squares], $V_3 = 13.3 \pm 0.8$ mV [triangles]) (V_1 versus V_2, $P < 0.01$; V_2 versus V_3, $P < 0.05$; unpaired t-test).

of action potentials. In most, if not all, spontaneously active cells, one or more clustered short-lasting deflections formed a 'foot' that raised membrane potential to spike threshold (Fig. 4A,B). When cells were hyperpolarized by injecting negative current at potentials around −90 mV, membrane deflections still occurred, but they were ineffective at raising membrane potentials above the spike threshold (Fig. 4C).

The occurrence of bursts of spikes was related with a complex interplay between short-lasting depolarizations and longer depolarizing waves as follows. The onset of bursts coincided with the switch-on of medium-lasting waves. Short-lasting deflections of membrane potential sustained the triggering of spikes (Fig. 4B, arrows). Bursts usually ended with the occurrence of two to three short-lasting depolarizing deflections that coincided with both an aborted spike (Fig. 4B, arrowhead) and a slow repolarization to the resting membrane potential.

The presence of both medium- and long-lasting depolarizing waves raised the possibility that these two events were temporally organized in relation with one another in that medium-lasting waves appeared superimposed over long-lasting waves. This overlay between the three patterns of voltage fluctuations was particularly prominent in cells responsive to GRF ($n = 5$). Fig. 5A illustrates a typical GRF response with a complex but organized superposition of low-amplitude voltage deflections ranging from about 1 s (associated or not with the triggering of individual action potentials) to several minutes. Expanding time scales in Fig. 5B,C illustrate voltage waves of at least three distinguishable time frames.

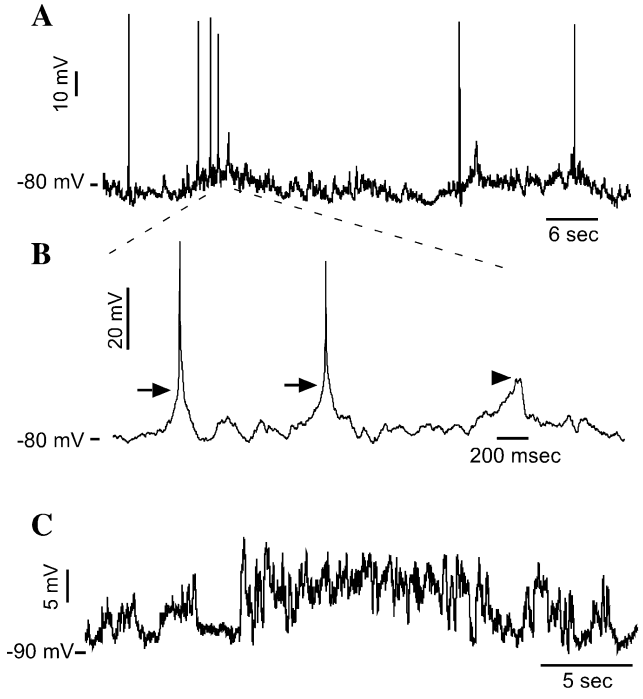

Fig. 4. Contribution of small-amplitude voltage fluctuations to spontaneous spike firing in situ. A: Arrangement of voltage fluctuations associated with the triggering of spontaneous action potentials. B: Expanded time scale of a portion of the patch-clamp recording shown in panel A. Arrows: approximate spike threshold. Arrowhead: aborted spike. C: Small-amplitude voltage fluctuations failed to promote spike firing when the membrane was hyperpolarized to potentials below the spike threshold.

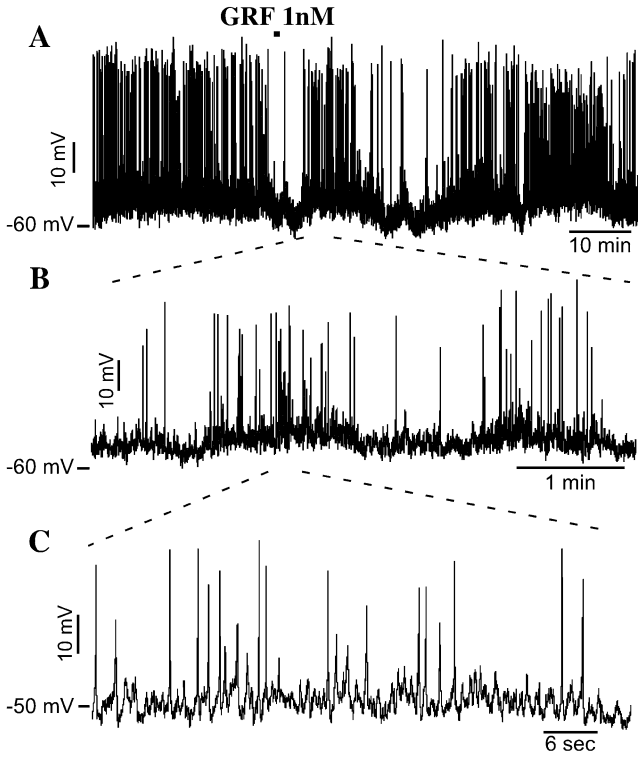

Fig. 5. Multimodal arrangement of both voltage fluctuations and action potentials in a secretagogue-stimulated cell. A: Typical electrical recording of a cell responsive to a local application of growth hormone-releasing hormone (GRF). B,C: Expanded time scales of prolonged bursts of action potentials, which occurred after GRF application. Note that small-amplitude voltage deflections were arranged on top of each other in an order based on fluctuation duration. This multimodal arrangement of voltage fluctuations dictated the burst patterning of action potentials, which occurred in response to GRF.

4. Discussion

This study provides evidence that pituitary endocrine cells possess in situ an intrinsic system of generating small-amplitude deflections in membrane potential. The comprehensive arrangement of these fluctuations allows pituitary cells to finely tune their electrical activity over a wide range of time scales under both unstimulated and agonist conditions.

Spike initiation depends in situ, at least in part, on the building-up of one or a few short-lived (≈1 s average), small-amplitude deflections in membrane potential that incrementally depolarize the membrane up to the action potential threshold. The occurrence of these short voltage fluctuations was high when cells fired action potentials at high frequency. It is also worth noting that summation of voltage fluctuations coincided with the termination of spike bursts. This probably reflects the presence of a complex interplay between voltage fluctuations and ion channels shaping action potentials, which continuously adapts the concurrent level of membrane excitability of pituitary cells in situ.

Another major finding of the present study concerns the multimodal arrangement of voltage fluctuations. Close inspection of long-term patch-clamp recordings revealed that short-lived fluctuations were often clustered on top of medium-lasting (≈10 s average) depolarizing waves. Moreover, the latter could also be clustered on top of longer (≈100 s average)

waves. This multimodal arrangement was particularly prominent in cells that responded to the GRF secretagogue. This opens the door to a new perception of electrical activity coding in endocrine pituitary cells, which, to our knowledge, was never reported in studies using cultured pituitary cells [4,11–17]. As spike discharges with distinct lengths could be associated with three temporally defined modes of depolarizing deflections/waves (see e.g. Fig. 5), it is conceivable that different functions could be simultaneously, but finely, modulated by these three patterns of bursting activity. The fact that our patch-clamp measurements were limited to up to 2 h recordings does not preclude that endocrine pituitary cells are also likely to display longer voltage waves. For instance, pituitary glands cultured ex vivo sustain circadian oscillations of clock gene expression [21]. Since clock gene rhythmicity governs circadian rhythms of electrical firing in the SCN [22–24], it would be worth investigating whether a circadian organization of electrical firing exists in pituitary cells.

We further explored whether spike firing linked to voltage fluctuations could be detected in acute pituitary slices issued from different species. They were present in pituitary cells from both two rodent (rat and mouse) and one non-rodent species (guinea-pig) [25]. Moreover, guinea-pig pituitaries display a parenchymal organization of well-defined cell cords that resemble those observed in human beings. This raises the possibility that voltage fluctuations form a widespread pituitary mechanism among mammalian species. This proposal should be carefully examined in case of abnormal functioning; in particular, tumoral cells from pituitary adenomas have been shown to display fairly regular 'pace-maker' electrical activity with little evidence of voltage fluctuations in culture [26,27].

In conclusion, the present results have marked a step forward in identifying the mechanisms that trigger electrical activity in the anterior pituitary, the endocrine gland that controls basic body functions such as growth and fertility. Because small-amplitude voltage fluctuations most probably trigger action potentials in situ, the present results should, therefore, renew interest in the mechanism(s) by which pituitary endocrine cells generate these fluctuations in both basal and agonist-stimulated conditions. Further investigating would help determine whether the multimodal coding of these fluctuations is an inherent property of individual cells or depends on the cell environment in the pituitary parenchyma (cell–cell communication(s) between distinct cell types, cell contacts with the extracellular matrix...). To do so, it would greatly help to identify live endocrine cell types tagged with e.g. selective expression of green fluorescent protein [20,28] together with the specific labeling of non-endocrine folliculostellate cells with a UV-excitable fluorescent dipeptide [29].

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