Synchronized Spontaneous Ca\(^{2+}\) Transients in
Acute Anterior Pituitary Slices*

(Received for publication, December 1, 1997, and in revised form, January 28, 1998)

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We investigated the organization of spontaneous rises in cytosolic free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) due to electrical activity in acute pituitary slices. Real-time confocal imaging revealed that 73% of the cells generated fast peaking spontaneous [Ca\(^{2+}\)]\(_i\) transients. Strikingly, groups of apposing cells enhanced their [Ca\(^{2+}\)]\(_i\) in synchrony with a speed of coactivation > 1,000 μm/s. Single-cell injection of Neurobiotin or Lucifer yellow labeled clusters of cells, which corresponded to excitable cells. Halothane, a gap junction blocker, markedly reduced the spread of tracers. Coupling between excitable cells was mainly homologous in nature, with a prevalence of growth hormone-containing cells. We conclude that spontaneously active endocrine cells are either single units or arranged in synchronized gap junction-coupled assemblies scattered throughout the anterior pituitary gland. Synchrony between spontaneously excitable cells may help shape the patterns of basal secretion.

Endocrine pituitary cells that release hormones from large dense core vesicles (LDCV) by calcium-mediated exocytosis exhibit spontaneous firing of action potentials. In cultured cell preparations, individual cells present asynchronous activity with different firing patterns (pace-making or bursting mode). When electrical recordings are combined with fluorescent monitoring of cytosolic free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)), single spontaneous spikes trigger transient rises in [Ca\(^{2+}\)]\(_i\), with characteristic features; a time to peak of less than 1 s and a return within a couple of seconds (1). The lag between the onset of the [Ca\(^{2+}\)]\(_i\) rise and exocytosis is also within a subsecond range (2, 3), sharing common features of stimulus-secretion coupling with neuroendocrine cells (e.g., chromaffin cells) and neurons, which release peptides from LDCV (4–6).

Although electrical activity has already been detected in the anterior pituitary gland, both in vivo and in tissue preparations (7–9), the dynamics and organization of [Ca\(^{2+}\)]\(_i\) rises associated with spontaneous action potentials have not yet been investigated at the tissue level. Based on the heterogeneous distribution of the five secretory cell types throughout the tissue (10), endocrine cells would display asynchronous firing so that the overall activity of each secretory type would simply reflect the average of single cell events. Cell regulation should mainly depend on the input of hypothalamic clocks, such as sequential release of growth hormone-releasing factor and somatostatin, which have been shown to pace growth hormone (GH) release (11). However, the gland disconnected from the hypothalamic inputs still shows pulsatile GH release (12), suggesting a synchronization of cellular signals within the tissue. With regard to the mechanisms accounting for synchronization in other tissues (13–18), two sources of cell-to-cell communication, not mutually exclusive, could be proposed. First, both endocrine and non-endocrine (foliculostellate) pituitary cells release various products (ATP, dopamine, and so forth), which locally act on neighboring cells (19–21). Second, gap junctions present in the anterior pituitary (9, 22–24) may allow both metabolic and electrical coupling between connected cells.

To study the behavior of spontaneously active cells within the adenohypophysis, we measured the multicellular patterns of spontaneous [Ca\(^{2+}\)]\(_i\), rises in acute slices of guinea pig pituitary, which preserved tissue structure (25). Real time confocal laser microscopy with the Ca\(^{2+}\)-sensitive fluorescent dye fluo-3 offers a sensitive method for optical recording of the fast peaking [Ca\(^{2+}\)]\(_i\) transients due to spontaneous action potentials. By visualizing the multicellular profiles of [Ca\(^{2+}\)]\(_i\), activity in these slices, we detected clusters of spontaneously active endocrine cells, which were scattered throughout the anterior pituitary. An abstract of a preliminary account of these results has already been presented (51).

EXPERIMENTAL PROCEDURES

Tissue Slice Preparation.—Acute pituitary slices were prepared according to previously reported methods (26). Briefly, the pituitary gland was removed from 4–8-week-old female guinea pigs (OCF-DH albino) that had been killed by decapitation after pentobarbital anesthesia. After keeping the gland in ice-cold saline for 2 min, it was glued onto an agarose cube and transferred to the stage of a vibratome (Microslice®, DTK-1000, D.S.K, Dosaka EM Co., Ltd., Kyoto, Japan). Coronal slices of 150-μm thickness were then cut with a razor blade and transferred to a storage chamber thermostated at 32 °C, containing Ringer’s saline (in mM): 125 NaCl, 2.5 KCl, 1.0 CaCl\(_2\), 1.25 NaH\(_2\)PO\(_4\), 26 NaHCO\(_3\), 12 glucose, and buffered to pH 7.4. The saline was continuously bubbled with carbogen (95% O\(_2\), 5% CO\(_2\)). As reported for the slices of the intermediate lobe (26), slices of the anterior lobe were suitable for patch-clamp recordings and Ca\(^{2+}\) signal measurements immediately after cutting. Slices were viable up to 8 h, as seen by the presence of spontaneous [Ca\(^{2+}\)]\(_i\) elevations. To achieve optical and/or electrophysiological recordings, pituitary slices were transferred to a recording chamber attached to the stage of an upright microscope fitted with differential interference contrast optics (Axioskop FS, Zeiss, Le Pecq, France) and continuously superfused with Ringer’s saline at 30 °C.

Confocal Microscopy.—Fast spontaneous [Ca\(^{2+}\)]\(_i\) transients were routinely measured by a real time (30–450 frames/s) confocal laser scanning microscope equipped with an ArKr laser (Odyssey XL with InterVision 1.4.1 software, Noran Instruments Inc., Middleton, WI). Cells were viewed with a 63 × 0.9 numerical aperture achroplan water immersion objective lens (Zeiss). Various thicknesses of confocal images were viewed.
was obtained by selecting different detection slits. The larger slit (100 μm) was used for [Ca\textsuperscript{2+}] signals, giving bright images with a 3.1-μm axial resolution. When cells were subsequently loaded with Lucifer yellow (see below), confocal images were acquired with a 25-μm slit, which provided an axial resolution of ~1.3 μm. Slices were loaded with the fluorescent dye Lucifer yellow. Perforation of the patch electrode was performed using a PicoPump 30 (World Precision Instruments, Sarasota, FL). Cells were viewed with a 63× objective. Images were acquired with the confocal microscope as described above. Cells were viewed with a 63×1.4 numerical aperture plan-apochromat with a 15-μm detection slit (0.65-μm axial resolution).

**Test Substances—**Drugs were pressure-ejected from an extracellular micropipette (tip diameter 2–5 μm), the tip of which was positioned in the vicinity of the recorded cells. The concentration reported are those in the pressure pipette. Lucifer yellow, somatostatin-14, and the calcium channel blocker CdCl\textsubscript{2} were used from Sigma. The gap junction blocker halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) was purchased from FluKo. To obtain Ca\textsuperscript{2+}-free solution, CaCl\textsubscript{2} was omitted from, and 5 mM EGTA was added to modified Ringer’s saline. The Neurobiotin tracer was purchased from Vector Laboratories (Bayside S.A., Compiegne, France).

**RESULTS**

Experiments were performed in coronal slices (150-μm thickness) from pituitary of 4–8-week-old female guinea pigs. Slices were loaded with fluo-3/AM by bath application of the CA\textsuperscript{2+} indicator, which produced widespread staining of the first and second layers of cells on the slice surface. When visualized with epifluorescence under the upright microscope, numerous cells within each field exhibited spontaneous changes in fluo-3 emission, reflecting rises in [CA\textsuperscript{2+}], (31). Time-lapse optical sequences of the cells showing spontaneous [CA\textsuperscript{2+}], rises were then recorded with fast scanning confocal imaging (120 images/s with averaging 4 frames) during different experimental protocols.

**Presence of Spontaneously Coactive Cells in Acute Pituitary Slices—**In most slices, one to several groups of adjacent active cells which fired synchronously were observed, as visualized at first using the epifluorescence part of the microscope. Real-time optical imaging revealed that these clusters of synchronous cells coexisted with asynchronous neighboring cells as illustrated in Fig. 1A. The top left image shows a section in which four fluo-3-loaded cells could be observed in the same optical section. The montage of consecutive optical slices depicts a time series of fluo-3 emission frames encoded in pseudocolours (from blue to red with [Ca\textsuperscript{2+}] increasing). The three bottom cells fired spontaneous fast-peaking [Ca\textsuperscript{2+}] transients. The plots of relative fluo-3 emission changes show that two cells paced their [Ca\textsuperscript{2+}], in synchrony, whereas the third one had its own rhythm. With only the first pair of synchronized [Ca\textsuperscript{2+}] transients, it seems likely that the red-circled cell became active before the green-circled one while the lag between the following pairs of [Ca\textsuperscript{2+}] transients was indistinguishable under the time resolution used in these experiments.
FIG. 1. Spontaneous synchronized [Ca$^{2+}$], transients due to Ca$^{2+}$ entry in cell multiplets. Fast cytosolic Ca$^{2+}$ transients were recorded using real time scanning laser confocal imaging (120 images/s with averaging 4 frames). A, upper part, time-lapse optical sequences illustrating the generation of spontaneous [Ca$^{2+}$], transients in three adjacent cells. Each frame (66 ms between frames) consisted of an average of two successive images. The color circles highlight the area of each cell used to monitor fluo-3 emission changes. Fluo-3 emission frames were encoded in pseudocolors (from blue to red with [Ca$^{2+}$], increasing). Lower part, plots of relative fluo-3 emission changes showing that the red-circled and green-circled cells paced their [Ca$^{2+}$], synchronously, whereas the blue-circled one had its own rhythm. B, example of synchronized spontaneous [Ca$^{2+}$], transients recorded in a multiplet (four coactive cells in the same optical section). C, a 10-s local application of a Ca$^{2+}$-free solution containing 5 mM EGTA reversibly blocked synchronized [Ca$^{2+}$], transients. Inset, expanded time scale showing that the first [Ca$^{2+}$], transients still occurred in synchrony after washout.

Both synchronous and asynchronous cells presented spontaneous [Ca$^{2+}$], transients with roughly similar kinetics (time to peak = 150.6 ± 6.8 ms, n = 141), patterns (pace-maker or bursts) and firing frequency (0.54 ± 0.07 Hz, range from 0.1 to 2.4 Hz, n = 43). No difference was noted with regard to the location of synchronized cell clusters within coronal slices. In addition, spontaneously coactive cells were also detected in sagittal slices (n = 4) suggesting a lack of preferential orientation for the spread of coactivation among spontaneously active cells. Confocal imaging routinely enabled us to observe doublets of coactive cells in single optical sections. However, multiplets with three to four coactive cells were occasionally encountered (Fig. 1B).

The kinetic features of spontaneous [Ca$^{2+}$], transients observed in acute pituitary slices along with previous [Ca$^{2+}$], data obtained in cultured pituitary cells (1, 32) suggest that these [Ca$^{2+}$], fluctuations resulted from voltage-gated Ca$^{2+}$ entry. We therefore investigated their extracellular Ca$^{2+}$ dependence. Local application of a Ca$^{2+}$-deprived solution reversibly blocked synchronous [Ca$^{2+}$], transients (Fig. 1C, n = 5) as well as asynchronous [Ca$^{2+}$], fluctuations (n = 24). A Ca$^{2+}$-containing Ringer’s saline (0.5 mM CdCl$_2$) ejected in the vicinity of cells had a similar effect on both synchronous (n = 3) and asynchronous [Ca$^{2+}$], transients (n = 8, data not illustrated). Interestingly, the first [Ca$^{2+}$], transients still occurred synchronously after washout (Fig. 1C, inset), suggesting that blockade of Ca$^{2+}$ entry did not alter the coactivation spread. When [Ca$^{2+}$], monitoring was combined simultaneously with recordings of membrane potential using the perforated patch-clamp technique, the triggering of a single action potential was sufficient to elicit a rapid increase in [Ca$^{2+}$], with kinetics (time to peak = 182.4 ± 25.1 ms, n = 18) similar to those of spontaneous [Ca$^{2+}$], transients recorded in cells not subjected to patch-clamping (p > 0.05, data not shown).

Dye Diffusion between Spontaneously Coactive Cells—Different communication mechanisms, either electrical or biochemical, could explain the synchronization of spontaneously active cells. Since coactivation always occurred between apposing cells of finite clusters, the synchronization signal should be restricted to coactive cell-cell boundaries, but not extensively diffused to neighboring asynchronous cells. Since gap junctions were described in the anterior pituitary (22), we carried out dye coupling experiments with a low molecular weight fluorescent dye LY (457 Da). The tracer was injected through a sharp microelectrode into single cells belonging to synchronized clusters. Fig. 2A illustrates an example of two neighboring cells exhibiting synchronized spontaneous [Ca$^{2+}$], transients. After fluo-3 emission measurements, cell labeled 2 was impaled with LY (4% in 150 mM LiCl). A few seconds later, cell labeled 1 was also stained with LY, indicating the LY diffusion from the
impaled cell to the coupled partner. No diffusion was observed in other adjacent cells \( (n = 21) \). Moreover, LY diffusion was restricted to single impaled cells, which spontaneously fired with their own rhythm \( (n = 14) \). These data strongly suggest that gap junctions could ensure the spread of coactivation between excitatory pituitary cells. Since large molecules \( (\approx 1,000 \text{ Da}) \) usually do not permeate through gap junctions, further experiments were conducted with large molecular weight dextran conjugates. Cells were randomly loaded with Neurobiotin, a low molecular mass dye \( (323 \text{ Da, 1\% in internal patch pipette solution}) \) and dextran Texas Red \( (3,000 \text{ Da, 2 mg/ml}) \), by diffusion for 10–30 min. In two out of seven clusters, patched cells contained both markers, whereas coupled cells were labeled with Neurobiotin only \( (\text{data not shown}) \). In the others, no dye diffusion was observed.

When LY or Neurobiotin were injected into cells not subjected to \([Ca^{2+}]\), imaging, the tracers diffused from the impaled cell to neighboring cells in 47\% of the clusters tested \( (n = 57/121) \). The extent of dye coupling, however, did not usually exceed five to six cells, as seen by three-dimensional reconstruction of optical slices \( (\text{data not shown}) \). Similar experiments were further conducted in presence of the gap junction blocker halothane \( (33, 34) \). In pituitary slices bathed for 15–60 min in Ringer’s saline saturated with 3 mM halothane, the appearance of dye-coupled cells was markedly reduced. \( *p < 0.05 \) as compared with control values. The reversibility of halothane effects was checked by placing treated slices in a halothane-free solution for 1–3 h. D, uncoupling effect of halothane \( (3 \text{ mM}) \) leading to asynchronous \([Ca^{2+}]\), transients. Synchronized \([Ca^{2+}]\), transients were first recorded in two coactive cells. Halothane was then bath-applied for 3 min before the subsequent \([Ca^{2+}]\), monitoring.

**Fig. 2.** Gap junction-mediated dye diffusion between spontaneously synchronized cells. A, two neighboring cells exhibiting synchronized \([Ca^{2+}]\), transients. After fluo-3 emission measurements, cell labeled 2 was injected with LY \((4\% \text{ in } 150 \text{ mM LiCl})\). After a few seconds, LY diffused into the coupled partner \( (\text{cell labeled 1}) \). No diffusion was observed in other adjacent cells. B, in asynchronous cells, LY diffusion was restricted to the impaled cell \( (\text{cell labeled 2}) \). C, in pituitary slices bathed for 15–60 min in Ringer’s saline saturated with 3 mM halothane, the appearance of LY-coupled cells was markedly reduced. \( *p < 0.05 \) as compared with control values. The reversibility of halothane effects was checked by placing treated slices in a halothane-free saline for 1–3 h. D, uncoupling effect of halothane \( (3 \text{ mM}) \) leading to asynchronous \([Ca^{2+}]\), transients. Synchronized \([Ca^{2+}]\), transients were first recorded in two coactive cells. Halothane was then bath-applied for 3 min before the subsequent \([Ca^{2+}]\), monitoring.

**Spreading of Coactivation Is Higher than 1,000 \mu m/s**—Spread of coactivation through gap junctions can be due to either simple diffusion of \([Ca^{2+}]\) from a trigger cell to coupled cells or electrical coupling between these cells. Since these two mechanisms are associated with distinct speeds of propagation, \(x,t\)-series line scans were performed to estimate the speed of the wave of coactivation. A single horizontal line crossing synchronized cells was continuously scanned over 2,133 ms \((4.4 \text{ ms/line, 480 lines})\). Fig. 3A shows each line displayed in time along the \(y\) axis. The time course of the fluorescence changes in synchronized cells revealed a 5.6-ms delay between the onset of spontaneous \([Ca^{2+}]\), transients. Taking into account the distance between cell centers, the average speed of the coactivation wave from the trigger cell to the other was 1,056 \pm 112 \mu m/s \((n = 40, 5 \text{ different coactive clusters})\). Interestingly, the calculation of the delay between \([Ca^{2+}]\), transients within coactive cell assembly revealed that the trigger cell could become the responding cell and vice versa during recordings \( (\text{four out of five clusters, Fig. 3B})\).

In parallel experiments, an external voltage stimulation \( (6 \text{ Hz, } n = 14, \ p > 0.05) \) of spontaneous \([Ca^{2+}]\), transients. In the other cells, halothane, however, reduced \([Ca^{2+}]\), spiking. The reversibility of halothane effects was checked by placing treated slices in a halothane-free saline for 1–3 h. Under these conditions, the percentage of dye-coupled cells recovered to control level \( (n = 40, p > 0.05) \). Fig. 2D shows the uncoupling effect of halothane \( (3 \text{ mM, 3–5 min bath application}) \) on previously coactive cells \( (n = 5) \). In addition, LY injection in one of these cells failed to label its neighboring cells. Taken together, these data strongly suggest that the spread of synchronization could be mediated by gap junctions.
The trigger and responding cells at the first pair of synchronized [Ca^{2+}] transients were defined as cells labeled 1 and 2, respectively. To calculate the delay, the starting point of each [Ca^{2+}] transient was determined by the intersection between two linear regressions calculated from the base line and the raising phase of [Ca^{2+}] transients.

V, 2 ms) was delivered from a micropipette touching one cell at the slice surface. Such a stimulation induced a fast [Ca^{2+}] rise which could propagate to one or two adjacent cells in the same focal plane (n = 9, data not shown). The speed of propagation calculated from x-t-series data (1,639 ± 613 μm/s, n = 4 clusters) did not differ from that found between spontaneously synchronized cells (p > 0.05).

Prevalence of Homologous Coupling between GH-containing Coactive Cells—Earlier studies carried out in pituitary cells isolated from the tissue have revealed that spontaneous electrical activity and ensuing [Ca^{2+}] transients were mainly observed in cells containing either GH or prolactin (35). Since somatotrophs are the dominant secretory cell type in the anterior pituitary, we therefore investigated whether coactive cells have been extensively described, namely in somatotrophs (∙∙∙). A combination of real time optical imaging with dye-coupling studies enabled us to observe a fast speed of coactivation (>1,000 μm/s) that involves cell-to-cell communication via gap junctions. In contrast, other cells which spontaneously display asynchronous [Ca^{2+}] transients are never dye-coupled to neighboring cells.

The discovery of coactivation of spontaneously active pituitary cells has marked a new step in our knowledge of how endocrine cells secreting from LDCV interact with one another in the absence of any stimulus. Although domains of spontaneously coactive cells have been extensivly described, namely in the brain (16, 17, 36, 37), synchronization between excitable

DISCUSSION

Our experiments describe for the first time the multicellular [Ca^{2+}] rises, which spontaneously occur in acute slices from anterior pituitary. This spontaneous [Ca^{2+}] activity is spatially organized within the tissue into small groups of excitable cells that pace their [Ca^{2+}] synchronously. Combination of real time optical imaging with dye-coupling studies enabled us to observe a fast speed of coactivation (>1,000 μm/s) that involves cell-to-cell communication via gap junctions. In contrast, other cells which spontaneously display asynchronous [Ca^{2+}] transients are never dye-coupled to neighboring cells.

FIG. 3. Coactivation speed is higher than 1,000 μm/s. A, left panel, using the laser line (x-t) scanning mode, a single horizontal line (marked with arrows) crossing coactive cells was continuously scanned over 2,133 ms (4.4 ms/line, 480 lines). Right panel, the time course of the fluorescence changes in synchronized cells showed the delay between the onset of spontaneous [Ca^{2+}] transients. B, in another coactive cell domain, the trigger cell changed from one to the other during the recording. The histogram illustrates the coactivation delay from cell labeled 1 to cell labeled 2 (solid bars) and vice versa (open bars) (n = 18). The trigger and responding cells at the first pair of synchronized [Ca^{2+}], transients were defined as cells labeled 1 and 2, respectively. To calculate the delay, the starting point of each [Ca^{2+}], transient was determined by the intersection between two linear regressions calculated from the base line and the raising phase of [Ca^{2+}], transient.

FIG. 4. Prevalence of homologous coupling between growth hormone-containing synchronized cells. Upper panel, transmitted light (TL) image of a field of cells; LY diffusion into two synchronized cells. Immunostaining (GH) after slice fixation. Time-lapse optical sequences of the two GH cells showing synchronized spontaneous [Ca^{2+}], transients. Lower panel, plots of relative fluo-3 emission changes in the two GH-containing cells suggests that homologous coupling could at least involve subsets of spontaneously active somatotrophs. Somatostatin, a native inhibitor of GH release, reversibly blocked spontaneous [Ca^{2+}], transients issued from both synchronous and asynchronous somatotrophs (n = 6 and 13, respectively, data not shown). It should be noted that clusters of synchronized cells composed of both GH-positive and -negative cells were also encountered (5 out of 28 clusters). The remainders were GH-negative (9 out of 28).
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endocrine cells has only been reported in secretagogue-stimulated cells, such as beta cells from pancreatic islets exposed to high glucose levels. The latter are electrically silent at rest (i.e. at low glucose concentrations), while they display synchronized bursts of Ca\(^{2+}\)-driven action potentials only in response to their fuel secretagogue (38, 39).

In the brain, two distinct mechanisms support the spread of coactivation between spontaneously excitable cells. Synaptic transmission synchronizes neuronal activity in many brain areas (15), whereas coupling through gap junctions underlies local coactivation in, e.g. neocortex neuronal domains (16). Our results strongly suggest that gap junctions cause coupling of excitable pituitary cell subsets and thereby allow the synchronization of spontaneous [Ca\(^{2+}\)], transients in these assemblies of cells. Two observations concur with this proposal. Cell injection of small tracers (LY or Neurobiotin) results in the selective labeling of coactive cells and halothane, a gap junction blocker, markedly lowers the appearance of dye coupling without affecting the time course of spontaneous [Ca\(^{2+}\)], transients. Since all these studies have been done in slices, the relevance of these findings in vivo is yet unknown. Nevertheless, we and others have already observed LY transfer between unidentified pituitary cells in both neonatal rat pituitary slices maintained in long term (≥1 month) organotypic culture (9) and rat hemipituitaries (24). Ultrastructural and immunohistochemical studies have also demonstrated the presence of gap junction plaques and the expression of two connexin types (Cx26 and Cx43) in the anterior pituitary (22, 40, 41). Altogether, this strongly suggests that cell-to-cell calcium signaling mediated by gap junction communication is indeed present in the gland and is not a side effect of the acute slice preparation.

Line scanning mode experiments reveal that the speed of coactivation is higher than 1,000 µm/s. Although we do not rule out that Ca\(^{2+}\) or a metabolite (e.g. inositol 1,4,5-trisphosphate) can slowly diffuse through gap junctions in the cell assemblies (13, 14, 42), cell coactivation underlying synchronized [Ca\(^{2+}\)], transients is associated with a much faster and regenerative mechanism in the anterior pituitary. If single action potentials drive spontaneous [Ca\(^{2+}\)], transients, they should therefore act as a trigger for coactivation between gap junction-coupled cells. However, the occurrence of synchronized [Ca\(^{2+}\)], transients upon removing the blockade of Ca\(^{2+}\) entry suggests that maintenance of connectivity is not due to Ca\(^{2+}\) entry per se.

The mechanism driving the recruitment of coactive cells is tantalizing. What are the trigger cells? Given that external voltage stimulation causes a [Ca\(^{2+}\)] rise, which can propagate to adjoining cells, we suggest that electrical coupling mediates intercellular communication between excitable cells. However, the entrainment is not likely to be associated with the firing of fast-spiking cells which “chatter” slower cells (17), since the frequencies of [Ca\(^{2+}\)], transients are roughly similar in both synchronous and asynchronous cells. Interestingly, trigger cells can alternate with time within the coactive cell domain. The mechanisms that dictate the wide range of spiking patterns in the excitable pituitary cells remain hard to identify despite the fact that the voltage-gated channels that open during the action potential have been well characterized (35, 43). One would therefore assume that the stochastic occurrence of action potentials would continuously determine which cell triggers spikes in neighboring cells via gap junctions. Paracrine interactions may also play a significant role in the selection of trigger cells. Since single action potentials seem to be efficient in driving spontaneous [Ca\(^{2+}\)] transients is associated with a much faster and regenerative mechanism in the anterior pituitary. If single action potentials drive spontaneous [Ca\(^{2+}\)], transients, they should therefore act as a trigger for coactivation between gap junction-coupled cells. However, the occurrence of synchronized [Ca\(^{2+}\)], transients upon removing the blockade of Ca\(^{2+}\) entry suggests that maintenance of connectivity is not due to Ca\(^{2+}\) entry per se.

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The significance of spontaneously coactive cell domains in the anterior pituitary can be viewed in terms of [Ca\(^{2+}\)], signal and hormone secretion. Cells containing GH in their LDCV prevail in coactive cell assemblies. Interestingly, coactive GH-containing cells often coexist with nearby asynchronous GH cells. Hence, the patterns of GH release should depend on the integration of the exocytotic activities of both synchronous and asynchronous cells. Morphological studies have also suggested a polarized phenotype for GH cells in situ since the latter are mainly arranged in palisades alongside fenestrated capillaries (10). Thus, the concurrent level of secretory efficiency within the capillaries depends on the topographical distribution of the two distinct GH subsets within the columns of pituitary cells (so-called cell cords), which are separated by basal laminae, connective tissue and blood vessels (10).

An association between GH-positive and GH-negative cells was also encountered, suggesting that the intricate pattern of spontaneous coactivation may encode the trigger for releasing factors other than hormones. In our view, neurotransmitter-like factors found in LDCV (e.g. dopamine in somatotrophs and lactotrophs) (19) could represent putative candidates since their fast and evanescent actions on pituitary cells (46) could provide a fine tuning of nearby cell activities.

Besides a possible role for local control of secretion, coactivation of excitable endocrine cells may serve a more integrated function in the entire gland. This can require a substantial number of connections between synchronized groups. Can a mechanism account for close synchrony despite the apparent wide range of distances (and the presence of connective tissue) between spontaneously coactive areas in vivo? The episodic releases of hypothalamic secretagogues (e.g. GH-releasing factor) (11) might periodically alter the number of coupled cells by acting on gap junctions (47, 48). This is certainly plausible, but these connections might not be dense enough to allow synchronization across very distant coactive areas. An alternative scenario is that other cells might pace collective rhythms within the anterior pituitary gland. Folliculostellate cells would be good candidates since they form a cell network, which extends throughout the anterior pituitary gland (49). These cells are coupled by gap junctions (24, 49, 50) and communicate with endocrine cells via paracrine interactions (20) and gap junctions (23, 24). Extensive analysis of these two putative mechanisms would be of particular interest, insofar as long distance synchrony may be an important determinant for shaping the patterns of hormone release in the systemic circulation.

Acknowledgments—We are indebted to Drs. D. Debanne and O. Manzoni for critical reading, and M. Passama, A. Carrette, and R. Jaul for their excellent technical assistance. We thank the National Hormone and Pituitary Program and the National Institutes of Health, NIDDK, for the reagents.

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