

Rhythmic Bursts of Calcium Transients in Acute Anterior Pituitary Slices*

XAVIER BONNEFONT, JEROME FIEKERS, AUDREY CREFF, AND
PATRICE MOLLARD

INSERM U-469, Centre CNRS-INSERM de Pharmacologie-Endocrinologie, 34094 Montpellier Cedex 5, France; and the Department of Anatomy and Neurobiology, University of Vermont College of Medicine (J.F.), Burlington, Vermont 05405

ABSTRACT

Endocrine cells isolated from the anterior pituitary fire intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) transients due to voltage-gated Ca^{2+} entry. However, the patterns of $[\text{Ca}^{2+}]_i$ transients within the glandular parenchyma of the anterior pituitary are unknown. Here we describe, using real-time confocal laser microscopy, several spontaneous patterns of calcium signaling in acute pituitary slices prepared from male as well as cycling and lactating female rats. Forty percent of the cells demonstrated a spontaneous bursting mode, consisting of an active period of $[\text{Ca}^{2+}]_i$ transients firing at a constant frequency, followed by a rest period during which cells were either silent or randomly active. The remaining recordings from endocrine cells either demonstrated random $[\text{Ca}^{2+}]_i$ transients or were silent. These rhythmic bursts of

$[\text{Ca}^{2+}]_i$ transients, which required extracellular calcium, were detected in lactotrophs, somatotrophs, and corticotrophs within the acute slices. Of significance was the finding that the bursting mode could be adjusted by hypothalamic factors. In slices prepared from lactating rats, TRH recruited more bursting cells and finely adjusted the average duty cycle of $[\text{Ca}^{2+}]_i$ bursts such that cells fired patterned bursts for approximately 70% of the recording period. Eighty-six percent of these cells were lactotrophs. Thus, the rhythmic $[\text{Ca}^{2+}]_i$ bursts and their tuning by secretagogues may provide timing information that could encode for one or more cellular functions (*e.g.* exocytosis and/or gene expression) critical for the release of hormones by endocrine cells in the intact gland. (*Endocrinology* 141: 868–875, 2000)

OVER THE LAST decade, it has become evident that the triggering of intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) transients due to voltage-gated Ca^{2+} entry is critically important for the control of endocrine cell function in cultured or acutely dissociated cells from the anterior pituitary (1–3). Despite this wealth of information, one key question remains: what pattern of $[\text{Ca}^{2+}]_i$ transients is a reliable unit of information (4)? Individual isolated cells display spontaneous $[\text{Ca}^{2+}]_i$ transients over a broad repertoire of patterns: random spiking, pacemaker, and occasionally short duration clusters of $[\text{Ca}^{2+}]_i$ transients (2–5). One common view of single cell coding is that the rate of $[\text{Ca}^{2+}]_i$ spiking is the important variable; that is, endocrine cells simply regulate in an up or down fashion the frequency of $[\text{Ca}^{2+}]_i$ transients depending on the environment of hypothalamic factors (2, 3, 5). This is at variance, however, with the well documented evidence that groups of $[\text{Ca}^{2+}]_i$ spikes can accurately encode information only if they are intermittently interrupted by rest periods (6–12). It is also unknown whether the pattern of $[\text{Ca}^{2+}]_i$ transients observed in isolated anterior pituitary cells has a functional meaning compared with that in endocrine cells present in their native environment (4).

The apparent requirement for cell to cell associations in the intact tissue to retain certain aspects of calcium signaling patterns (13–15) prompted us to examine calcium signaling in a preparation that preserves both the architecture of the pituitary glandular parenchyma and the occurrence of spontaneous $[\text{Ca}^{2+}]_i$ firing (4). Recently, we reported that a large proportion of endocrine cells in acute slices from female guinea pigs exhibited *in situ* spontaneous $[\text{Ca}^{2+}]_i$ transients due to electrical activity, which fired either asynchronously in cells of the same field or synchronously in neighboring cells probably coupled by gap junctions (16). However, the limited length of optical $[\text{Ca}^{2+}]_i$ recordings (30 sec to 2 min) did not allow us to discriminate distinct spiking patterns (16). Here, the improvement of fluorescent $[\text{Ca}^{2+}]_i$ recordings over long time scales together with numerical methods for pattern analysis has enabled us to identify a special processing of $[\text{Ca}^{2+}]_i$ transients infrequently observed in isolated single cells. This pattern occurred spontaneously in about 40% of endocrine cells in slices prepared from male as well as cycling and lactating female rats. It was rhythmic and bistable in nature. Each cycle period was characterized by a burst during which $[\text{Ca}^{2+}]_i$ spike frequency was relatively high and constant and an interburst interval during which $[\text{Ca}^{2+}]_i$ spikes occurred randomly or were absent. The remainder of recordings from the endocrine cells demonstrated either sporadic $[\text{Ca}^{2+}]_i$ transients or were silent. We also discovered that the ability of cells to fire rhythmic $[\text{Ca}^{2+}]_i$ bursts could be regulated by hypothalamic factors. The secretagogue TRH 1) induced a bursting mode in cells that were previously either silent or randomly active and 2) finely adjusted the timing of burst generation in bursting cells without altering the average intraburst frequency of $[\text{Ca}^{2+}]_i$ transients.

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Address all correspondence and requests for reprints to: Dr. Patrice Mollard, INSERM U-469, Centre CNRS-INSERM de Pharmacologie-Endocrinologie, 141 rue de la Cardonille, 34094 Montpellier Cedex 5, France. E-mail: mollard@u469.montp.inserm.fr.

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Materials and Methods

Tissue slice preparation

Acute pituitary slices (250–300 μm thickness) were prepared according to previously reported methods (16). Briefly the pituitary gland was removed from 6- to 12-week-old Wistar rats (males, cycling females, or 8- to 14-day lactating females) and cooled to 2–4 C in gassed (5% CO_2 -95% O_2) Ringer's saline containing 125 mM NaCl, 2.5 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , 1.25 mM NaH_2PO_4 , 26 mM NaHCO_3 , and 12 mM glucose and buffered to pH 7.4. Acute slices were then prepared using a vibrating blade microtome (Leica VT 10005, Leica, Nussloch, Germany) and transferred to a humidified incubator until used for $[\text{Ca}^{2+}]_i$ recordings. Slices were viable for up to 10 h after preparation.

Confocal cytosolic calcium imaging

Before recordings, slices were transferred to a chamber attached to the stage of an upright microscope (Axioskop-FS, Carl Zeiss, Le Pecq, France) and continuously superfused with Ringer's saline at 32 C. Cells were viewed with a $\times 63$ (0.9 numerical aperture) water immersion objective lens (Carl Zeiss) and then exposed intermittently to 15 μM Oregon Green 488 BAPTA-1 acetoxymethyl ester (Molecular Probes, Inc., Eugene, OR; 3 sec every 20 sec for 30 min) delivered onto a cell field via a blunt micropipette (4). To measure in real-time the averaged changes in Oregon Green 488 BAPTA-1 emission in endocrine cells, a fast-scanning confocal microscope (Noran Odyssey XL, Noran Instruments, Inc., Middleton, WI) was used (16). The bright over time tool of the software package was applied to live images (120 images/sec with averaging every four frames). A 100- μm slit was used for $[\text{Ca}^{2+}]_i$ signals, giving bright images with a 3.1- μm axial resolution. Acquired data were processed for analysis with InterVision 1.5.1 (Noran) and Igor Pro 3.13 (Wavemetrics, Inc., Lake Oswego, OR) software. The effects of both dye leak and photobleaching due to laser illumination were attenuated by correcting the basal level over the recording time. The baseline of each recording was fitted according to the equation $F(t) = A + B e^{-Ct}$, where A, B, and C are parameters, F is the fluorescence level, and t is the time. The corrected level F_c was then obtained using the equation $F_c(t) = F(t) - B e^{-Ct}$. The correction, however, did not completely overcome the apparent decay of burst amplitudes that occurred during long lasting optical recordings. Because Oregon Green 488 BAPTA-1 is a single wavelength dye, its emission is a function of both intracellular Ca^{2+} and dye concentrations. $[\text{Ca}^{2+}]_i$ changes were, therefore, expressed as the F/F_{min} ratio, where F_{min} was the minimum fluorescence intensity measured after off-line correction of the basal level.

Burst analysis

Direct determination, during optical recordings, of the positions of bursts was occasionally problematic because it was difficult to accurately determine the limits of the bursts when $[\text{Ca}^{2+}]_i$ transients occurred randomly between two bursts. Therefore, to analyze our recordings as accurately as possible, we performed a frequency analysis of the profiles of $[\text{Ca}^{2+}]_i$ spiking (17–19). First, the plots of F/F_{min} ratio reflecting the $[\text{Ca}^{2+}]_i$ changes were transformed to exclude the contribution of the baseline noise. The plots of $[\text{Ca}^{2+}]_i$ were differentiated so that the baseline fluctuated around 0.0 (see Fig. 2B). Second, the time of occurrence of each sharp deflection above 0 and above a threshold (corresponding largely to the onset of each $[\text{Ca}^{2+}]_i$ transient) was represented by a vertical line. Bursts in which several $[\text{Ca}^{2+}]_i$ transients occurred were, therefore, represented by groups of closely spaced parallel lines (raster plots). The frequency of $[\text{Ca}^{2+}]_i$ transients measured using the interline intervals was then plotted as a function of time. Third, values above a threshold, determined as one fifth of the maximal frequency during the recording, helped identify the boundaries of individual bursts. Only bursts that consisted of a minimum of three separated $[\text{Ca}^{2+}]_i$ transients were included in the analysis as an individual burst. Two parameters were then used as reliable indicators of bursting behavior (8, 20): 1) the duty cycle, defined as the proportion of one cycle period (equal to a burst duration plus an interburst interval), in which the $[\text{Ca}^{2+}]_i$ bursting is on; and 2) the bursting activity quotient (Q; the ratio between the total duration of bursts and the duration of the recording), which corresponds to the average duty cycle.

Immunofluorescence

After the real-time calcium recordings, one cell not subjected to $[\text{Ca}^{2+}]_i$ monitoring was impaled with a microelectrode containing the fluorescent dye Lucifer yellow (4% in 150 mM LiCl), and an image was acquired with the confocal microscope. The slice was then fixed by two successive formaldehyde solutions and processed for immunolabeling as previously described (16). We used a number of polyclonal (rabbit or guinea pig) antibodies raised against each pituitary hormone and donated by Drs. Y. Tillet (Institut National de la Recherche Agronomique, Nouzilly, France) and A. F. Parlow (National Hormone and Pituitary Program, NIDDK). The rabbit and guinea pig primary antibodies were revealed by Texas Red-conjugated donkey IgG and Cy5-conjugated donkey IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), respectively. Immunoreactivity in endocrine cells was imaged with the confocal microscope as described previously (16). Cells were viewed with a $\times 63$ (1.4 numerical aperture) plan-apochromat objective lens (Carl Zeiss).

Test substances

Drugs were pressure ejected from an extracellular micropipette (tip diameter, 2–4 μm), the tip of which was positioned in the vicinity of the recorded cells. The concentrations reported were those in the pressure pipette. Puffer applications of Ringer's saline had no effect on $[\text{Ca}^{2+}]_i$ activity ($n = 12$). TRH, tetrodotoxin (TTX; a Na^+ channel blocker), nickel chloride (Ni^{2+} ; a low threshold Ca^{2+} channel blocker), and Lucifer Yellow were all purchased from Sigma (St. Louis, MO). To obtain a Ca^{2+} -free solution, CaCl_2 was omitted from and 5 mM EGTA was added to Ringer's saline.

Statistics

When the spike frequency was analyzed within bursts, the bursts were divided into five equal parts, leading to five successive groups of $[\text{Ca}^{2+}]_i$ transients. Differences between mean spike frequencies were assessed by using a paired ANOVA. Analysis of cell distribution was carried out by using a χ^2 test. Differences with $P < 0.05$ were considered significant. Numerical data are expressed as the mean \pm SD. All curve fits were performed with the analysis menu of Igor Pro 3.13.

Results

Evidence for an endogenous $[\text{Ca}^{2+}]_i$ bursting pattern in situ

Time-lapse optical sequences of single cells loaded with the fluorescent Ca^{2+} probe were recorded with fast scanning confocal imaging (120 images/sec with averaging every 4 frames) within the first and second cell layers of acute slices. Cells were preloaded with Oregon Green 488 BAPTA-1, a high absorbance, low quenching, Ca^{2+} -sensitive dye, which permitted the discrimination of the profiles of $[\text{Ca}^{2+}]_i$ activity during 5–30 min of optical recording. In all acute pituitary slice preparations ($n = 42$), we observed coherent bursts during which groups of spontaneous $[\text{Ca}^{2+}]_i$ transients occurred in a repeating manner (Fig. 1, A–C). Recordings from cells not exhibiting a bursting mode were either silent or randomly active (Fig. 1D). A number of variations were encountered in the patterns of the $[\text{Ca}^{2+}]_i$ bursts during the optical recordings. In the vast majority of bursting cells (called bursters), both $[\text{Ca}^{2+}]_i$ spike trains and interburst intervals were irregular in length (range, 15–240 sec; Fig. 1A; $n = 201$). A few $[\text{Ca}^{2+}]_i$ spikes were also detected during the interburst intervals. On rare occasions bursts were identified with nearly identical epochs of oscillatory activity that recurred on a regular basis (Fig. 1B; $n = 33$). Both regular and irregular patterned $[\text{Ca}^{2+}]_i$ bursts were blocked by external Ca^{2+} removal (in the presence of 5 mM EGTA; 11 of 11 cells;

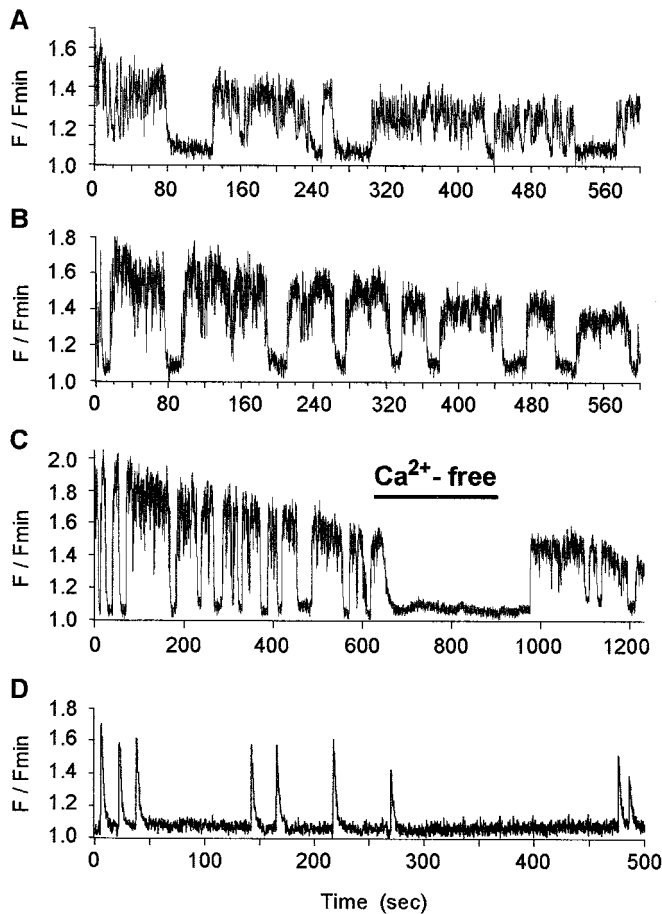


FIG. 1. Patterns of spontaneous $[Ca^{2+}]_i$ transients in acute rat anterior pituitary slices. Spontaneous $[Ca^{2+}]_i$ spiking in four different cells recorded with real-time confocal imaging. A, Irregular pattern of spontaneous $[Ca^{2+}]_i$ transients. B, $[Ca^{2+}]_i$ bursts separated by nearly identical silent periods. C, A local application of a Ca^{2+} -free solution containing 5 mM EGTA reversibly blocked the bursts of $[Ca^{2+}]_i$ transients. D, Random transient $[Ca^{2+}]_i$ spiking activity.

Fig. 1C) and markedly suppressed by either $50 \mu M Ni^{2+}$ (14 of 16 cells) or $5 \mu M TTX$ (8 of 15 cells; data not shown).

Another feature of the endogenous bursts in pituitary slices was the preservation of the $[Ca^{2+}]_i$ spike characteristics within the burst. To explore these characteristics, the relative changes in Ca^{2+} dye fluorescence (Fig. 2A) were differentiated, and peak values above a threshold were represented by raster plots (Fig. 2B). The spike frequency was then plotted as a function of time (Fig. 2C). No marked acceleration in spike frequency was noticeable within bursts ($P > 0.5$). Frequency analysis also permitted us to identify burst boundaries during optical recordings (Fig. 2C, values above a threshold denoted by the *dashed horizontal line*; see also *Materials and Methods*).

Distribution of spontaneously bursting cells in the anterior pituitary

How representative is the $[Ca^{2+}]_i$ burst pattern in the anterior pituitary tissue? Based on the criteria for burst identification described above, we observed that a large proportion of endocrine cells spontaneously fired bursts of $[Ca^{2+}]_i$

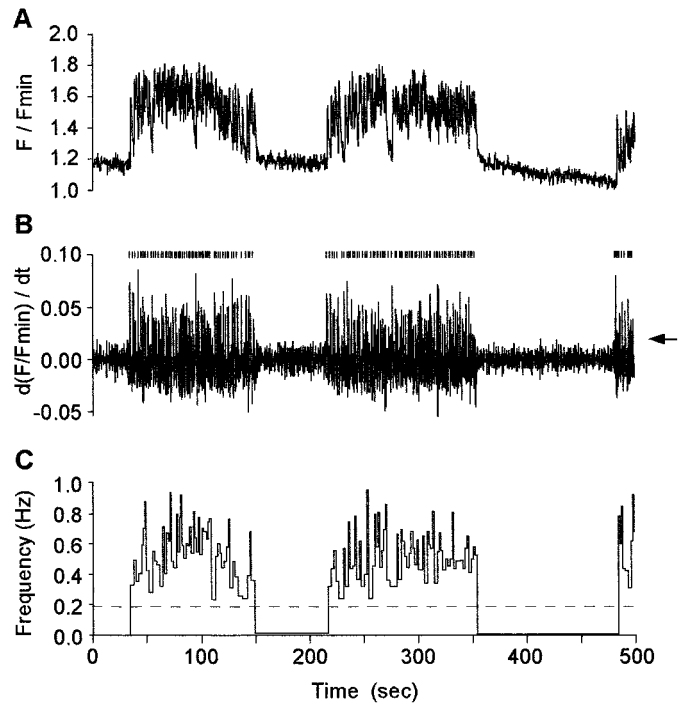


FIG. 2. Analysis of bursting activity. A, A representative plot of spontaneous $[Ca^{2+}]_i$ bursting activity. B, The occurrence of $[Ca^{2+}]_i$ bursts is represented by raster plots. To obtain the raster plots, the raw data shown in A are differentiated (*lower panel*), and each deflection above a threshold (indicated by the *arrow*) is depicted by a *vertical line* (*upper panel*). C, Instantaneous $[Ca^{2+}]_i$ spike frequency, calculated from the interline intervals, is plotted as a function of time. The burst boundaries are defined by the values above a threshold denoted by the *dashed horizontal line*.

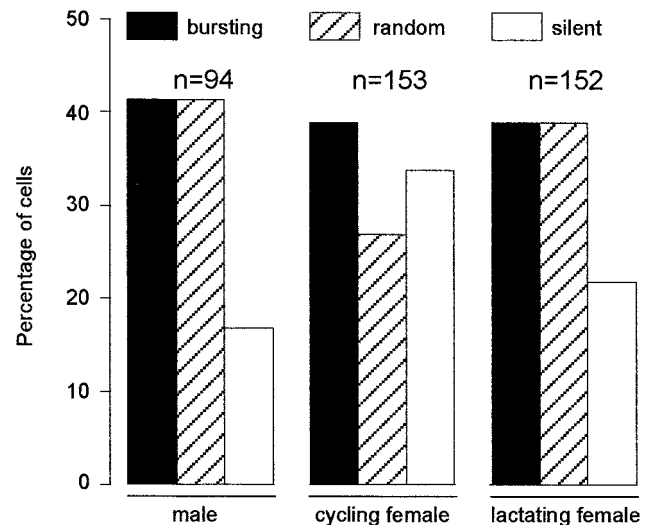


FIG. 3. Distribution patterns of spontaneous $[Ca^{2+}]_i$ activity. Percentage of cells that were either spontaneously active or silent in acute slices prepared from male, cycling, and lactating female rats, respectively. The number *above* each set of columns is the number of cells recorded. The distributions among the three animal preparations were significantly different ($P < 0.05$).

transients in male, cycling female, and lactating rats (Fig. 3). More strikingly, three endocrine cell types (lactotrophs, somatotrophs, and corticotrophs), which together represent the

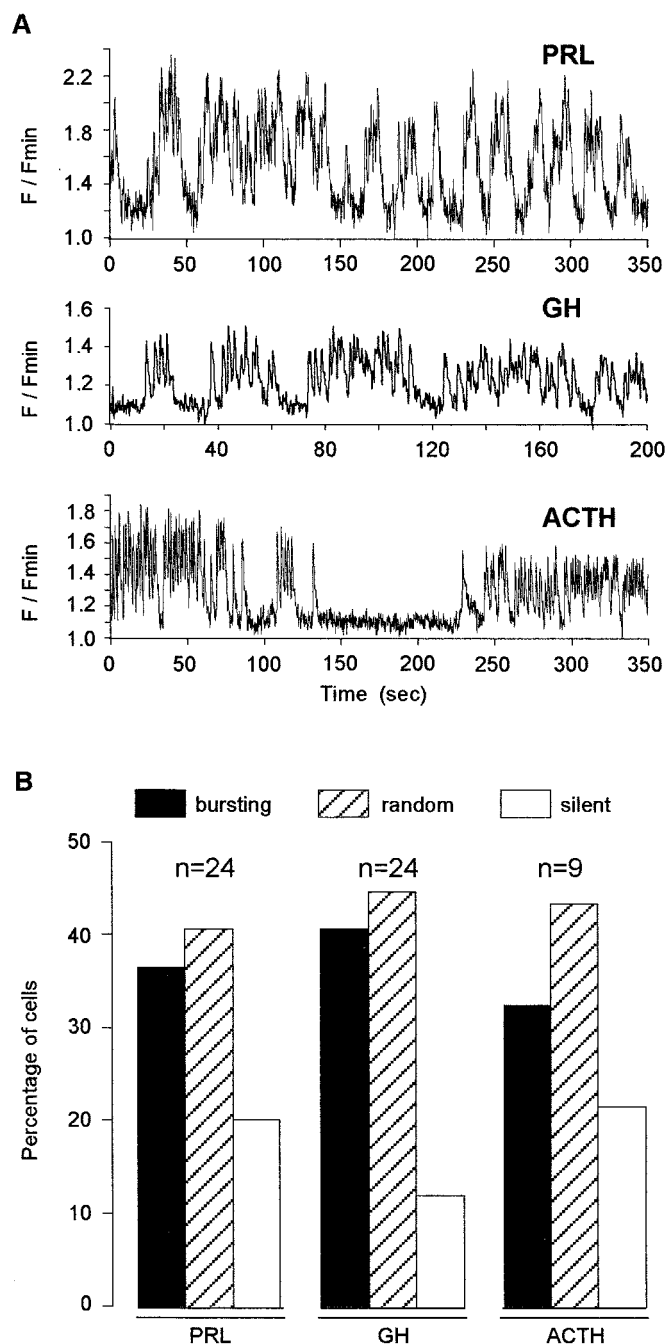


FIG. 4. Lactotrophs, somatotrophs, and corticotrophs spontaneously fire rhythmic $[Ca^{2+}]_i$ bursts. A, Patterned bursts of spontaneous $[Ca^{2+}]_i$ transients in three different cells that were immunoreactive for PRL (upper panel), GH (middle panel), and ACTH (lower panel), respectively. B, Percentage of cells that were either spontaneously active or silent in these three cell types.

majority of endocrine cells within the glandular parenchyma (21), displayed $[Ca^{2+}]_i$ bursts (Fig. 4A). The remaining cells were either randomly active or silent (Fig. 4B). In these three cell types, both burst and interburst durations were variable in length (up to several minutes). We do not preclude that the remainder of the cell types present in the adenohypophysis (gonadotrophs and thyrotrophs) display endogenous $[Ca^{2+}]_i$

bursts; we have not attempted to record from these cells because their density is low within the gland (22).

Endogenous bursters were detected at all levels of the gland studied (coronal or sagittal sections), suggesting a homogenous distribution throughout the parenchyma of the anterior pituitary. In addition, cells spontaneously fired rhythmic $[Ca^{2+}]_i$ bursts either asynchronously (*i.e.* at their own rhythm) or synchronously as coactive cell domains (two or three synchronized cells close to each other in a single plane of focus; $n = 23$) over long time scales (up to 15 min). These data corroborate our short term observations of synchronized $[Ca^{2+}]_i$ transients in endocrine cell ensembles from guinea pig pituitary slices (16). However, no preferential synchronization of bursters *vs.* the randomly active cells was observed during the optical recordings.

Setting the bursting pattern by TRH

As a number of cellular functions are dynamically orchestrated when a bolus of hypothalamic factors invades the glandular parenchyma, one would expect the bursting pattern to change upon the episodic arrival of a hypothalamic stimulus. To explore this, we investigated whether the secretagogue TRH modifies or even triggers patterned bursts in the anterior pituitary tissue. There are several lines of evidence that TRH promotes the firing of $[Ca^{2+}]_i$ transients in isolated lactotrophs and thyrotrophs (23) and perhaps in other cell types (24). We, therefore, performed these experiments using acute pituitary slices from lactating female rats, in which the density of lactotrophs is high (25). Baseline $[Ca^{2+}]_i$ activity was recorded for several minutes before the effects of a brief exposure to TRH (1 nM, 30 sec) were examined. As most spontaneous $[Ca^{2+}]_i$ bursts were irregular in nature, we measured the bursting activity quotient (Q) both before and after TRH application. During the control periods, Q was null in both silent and randomly active cells (Fig. 5, Aa and Ab, respectively); however, it was distributed over a wide range (14–98%) in endogenous bursters (Fig. 5, Ac–Ae).

The occurrence of patterned $[Ca^{2+}]_i$ bursts was the major effect of TRH action on $[Ca^{2+}]_i$ homeostasis in acute slices from lactating rats. The TRH effects lasted from 5–30 min. First, TRH switched on the bursting pattern in both silent and randomly active cells ($n = 3$ and 7, respectively; Fig. 5, Aa and Ab). Second, TRH reset the pacing in endogenous bursters. The effects of TRH depended on the bursting activity level of the cell before stimulation (Fig. 5, Ac–e and B). In cells showing an initial low Q value ($<50\%$), TRH caused a 2- to 3-fold increase in the Q value (Fig. 5Ac). When cells displayed an intermediate Q level (50–80%) before TRH, TRH finely readjusted their rhythmic pattern (Fig. 5Ad), whereas in highly active cells ($Q > 80\%$), TRH caused a decrease in the Q level (Fig. 5Ae). Another important finding was the unimodal distribution of Q values around a peak level of 67% in responsive cells regardless of whether they were bursting before TRH stimulation (Fig. 5C). Even though an acceleration of spike frequency was observed at the beginning of the first burst in some agonist-stimulated cells (17 of 63 cells), TRH had no effect on the average intraburst $[Ca^{2+}]_i$ spike frequency when examined over long time scales (Fig. 5D).

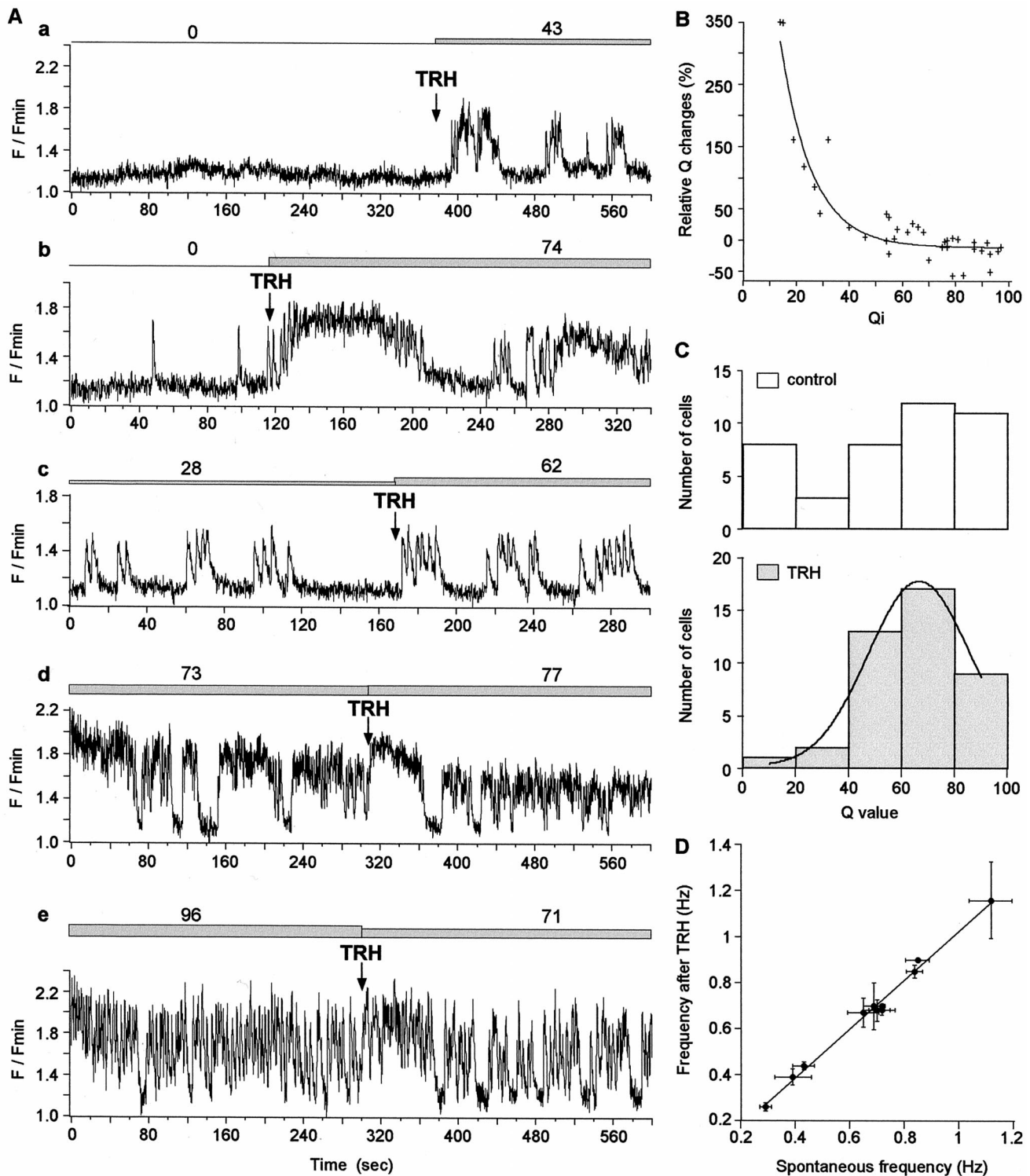


FIG. 5. Setting of bursting activity by TRH. A, The effects of 30-sec local applications of TRH (1 nM) on the $[Ca^{2+}]_i$ activity in five different cells. The Q values, which represent the proportion of time during which the cells displayed bursts, were calculated before and after TRH application (depicted by the numbers and thickness of horizontal lines on the top of raw data). Aa and Ab, TRH triggered bursts of $[Ca^{2+}]_i$ transients in two cells that were silent and randomly active, respectively. Ac–Ae, Changes in bursting activity induced by TRH in cells showing different Q values before agonist stimulation. These $[Ca^{2+}]_i$ bursts evoked by a low concentration of TRH were due to repetitive Ca^{2+} entry, as they were reversibly suppressed with external Ca^{2+} removal ($n = 25$). B, Each Q value calculated after TRH application is expressed as a relative change from the initial Q value (Q_i) and plotted as a function of Q_i . The solid line illustrates the apparent exponential decay of relative Q changes as Q_i increases (19). C, Effects of TRH on cells classified in five categories according to their respective initial Q values, which were binned for 20 Q-unit intervals. TRH reset the Q value with a single Gaussian distribution around a peak level of 67% regardless of whether the cells fired $[Ca^{2+}]_i$ bursts before agonist stimulation. D, Data illustrating the relationship between the frequencies of intraburst $[Ca^{2+}]_i$ transients before and after TRH application. The solid line shows a linear regression line fitted to the data ($y = 1.061x - 0.039$; $r = 99.5\%$; $n = 11$).

As more than 50% of endocrine cells are lactotrophs in the lactating rat anterior pituitary, we investigated whether TRH modulated the information processing of $[Ca^{2+}]_i$ transients in lactotrophs. To conduct this experiment, the PRL content of cells was postidentified by immunofluorescence (anti-PRL guinea pig antibody). Eighty-six percent of cells (33 of 39) that displayed a fine adjustment of $[Ca^{2+}]_i$ bursts in response to 1 nM TRH were lactotrophs (Fig. 6). The slices were also processed for TSH immunodetection (anti-TSH rabbit antibody). None of the bursters recorded was immunoreactive for TSH (data not shown).

Discussion

The data demonstrate that endocrine pituitary cells have the inherent capacity to spontaneously produce rhythmic bursts of $[Ca^{2+}]_i$ transients in their native environment. This mode of calcium signaling is expressed in a large proportion of endocrine cells of the anterior pituitary. We also observed a novel action of TRH on cells in the anterior pituitary, whereby the average duty cycle of $[Ca^{2+}]_i$ bursts is adjusted within a narrow range.

Long lasting optical recordings with the Ca^{2+} dye Oregon Green 488 BAPTA-1 allowed us to discern characteristic features of rhythmic $[Ca^{2+}]_i$ bursts that occurred in acute anterior pituitary slices. The pattern of the bursting mode was mostly irregular whenever endocrine cells fired $[Ca^{2+}]_i$ transients spontaneously or in response to TRH. Irregular bursts of an intracellular message are not unique to the anterior pituitary, as irregular trains of action potentials have been commonly observed in different brain areas such as the cortex and hippocampus (6). There are now several lines of evidence that this variability of patterned bursts *in situ* reflects the dynamic nature of cell activities on short time scales (6, 7, 26). The second striking characteristic of the bursting mode in endocrine pituitary cells was that the firing rate of $[Ca^{2+}]_i$ transients was sustained at a high average level (~ 0.7 Hz) throughout the bursts. This constant level of $[Ca^{2+}]_i$ spiking activity during the bursts resembles that of the pacemaker mode widely observed in isolated endocrine pituitary cells (3). However, in the intact glandular parenchyma, the $[Ca^{2+}]_i$ spike firing was episodically reduced (*i.e.* a drop in spike frequency) or even interrupted during the interburst

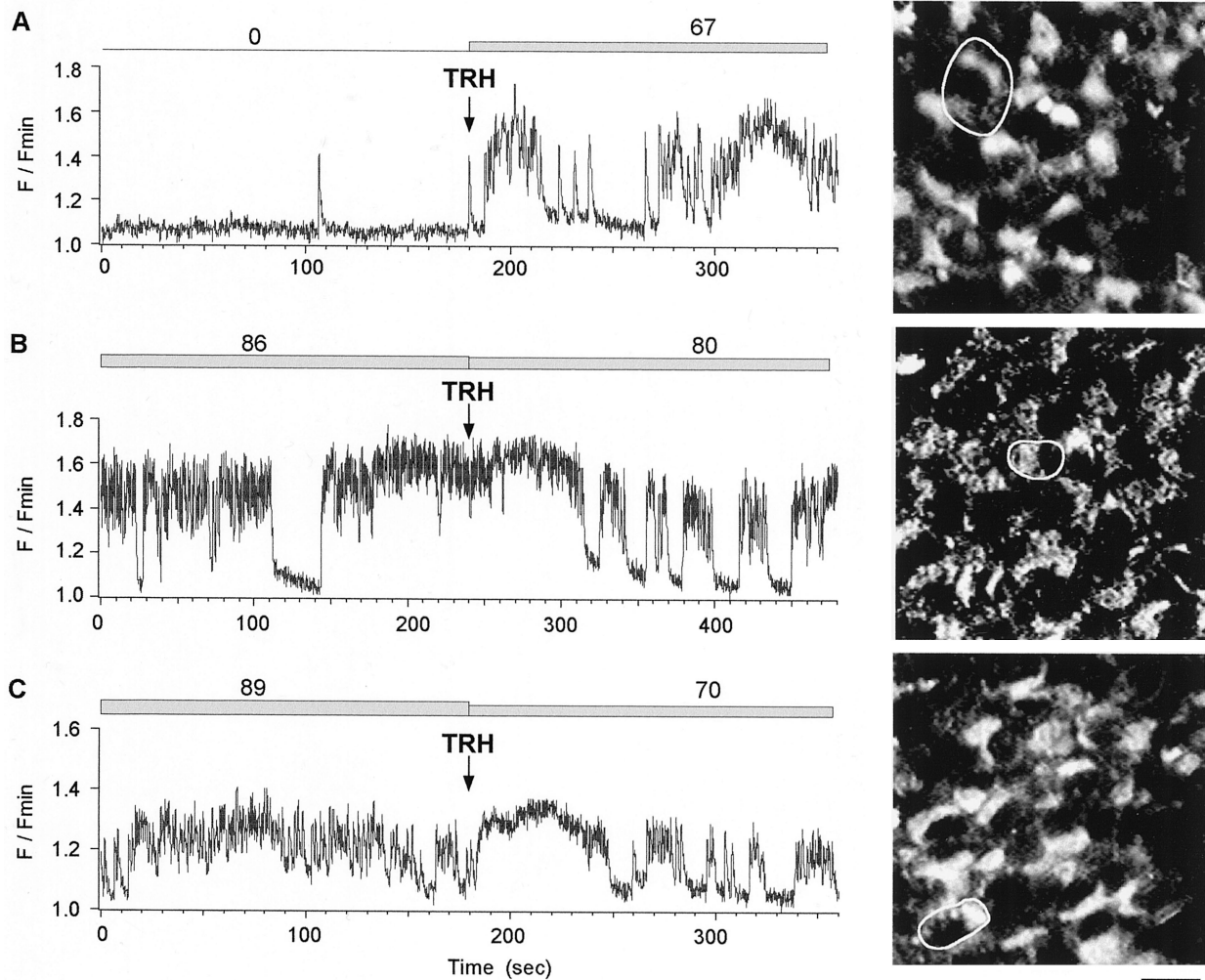


FIG. 6. TRH finely tunes a bursting behavior in lactotrophs. Three examples of TRH responses (left panels) in cells that were immunoreactive for PRL (circled cells in right panels). The Q values were calculated before and after TRH application (depicted by the numbers and thickness of horizontal lines on the top of raw data). The average Q value after TRH stimulation was $69.6 \pm 3.9\%$ ($n = 27$). Scale bar, 10 μm .

periods, which lasted up to several minutes. The $[Ca^{2+}]_i$ transients were probably due to Ca^{2+} -dependent action potentials because they were markedly suppressed upon either external Ca^{2+} omission or application of Na^+ and Ca^{2+} channel blockers (TTX and Ni^{2+} , respectively) (1–3). The mechanisms for generating the trains of action potentials that underlie the $[Ca^{2+}]_i$ bursts may differ among the cell types. They may involve various pacing currents, such as low threshold Ca^{2+} channel currents (27), pacemaker/bursting (If/Ih) currents (28), Ca^{2+} -dependent K^+ channel currents (29), and ATP-sensitive inward K^+ currents (30).

Although the intrinsic activity of cells seems to influence the occurrence of spontaneous $[Ca^{2+}]_i$ bursts, bursting is probably not a fixed property of endocrine cells. Two arguments support this assumption. First, neighboring bursting cells could be synchronized, suggesting that the trigger cell, but not necessarily the follower cell(s), expresses the intrinsic mechanisms for generating $[Ca^{2+}]_i$ bursts (16). Second, the ability to fire bursts can be regulated in cells (mainly lactotrophs) from lactating rats in response to TRH. This secretagogue switched on patterned bursts in some cells and harmonized the burst generation on long time scales so that rhythmic $[Ca^{2+}]_i$ bursts displayed an average duty cycle that was roughly identical for a large number of endocrine cells scattered throughout the glandular parenchyma. Burst setting by agonists has also been observed *in vivo* in hypothalamic vasopressin neurons, in which local vasopressin release could establish a special pattern of action potential discharges (19). This raises the possibility that one of the best stimuli for exciting, in a coherent fashion, a large number of peptide-releasing cells in their tissue context is to produce single cell rhythmic bursting patterns of intracellular messages with the same duty cycle.

The present work shows that TRH had no effect on average intraburst $[Ca^{2+}]_i$ spike frequency. This is at variance with studies performed on isolated pituitary cells in which a prolonged change in $[Ca^{2+}]_i$ spike frequency has been described as the major action of low concentrations of TRH (23, 31). The present results should, therefore, renew interest in the mechanism(s) by which TRH, and perhaps other stimuli (e.g. GH-releasing factor and CRF) (32–34), act on $[Ca^{2+}]_i$ signaling in endocrine cells from the anterior pituitary. It should be noted that although the majority of cells responding were lactotrophs (86%), other cells also displayed rhythmic $[Ca^{2+}]_i$ bursts upon agonist stimulation, suggesting an action of TRH (direct receptor stimulation and/or via paracrine interaction) on the bursting behavior of other cell types.

What are the functions of rhythmic $[Ca^{2+}]_i$ bursts? Although no information is available yet on the role of $[Ca^{2+}]_i$ bursts in the function of endocrine pituitary cells, the high efficiency of bursts of $[Ca^{2+}]_i$ spikes to trigger exocytosis in cells releasing peptides and catecholamines from large dense core vesicles has already been demonstrated (9–12). In addition, regulation of rhythmic $[Ca^{2+}]_i$ bursts can optimize the timing and amount of hormone release once they are paced with adequate rest periods (10–12), such as in lactotrophs upon TRH stimulation in lactating rats. Finally, cell to cell mechanisms (e.g. gap junction-mediated cell synchronization and paracrine factors) (16, 35, 36) might help coordinate the tempo of single cell hormone secretion due to $[Ca^{2+}]_i$ bursts

throughout the glandular parenchyma (37, 38). Further experiments in which $[Ca^{2+}]_i$ monitoring will be combined with single cell detection of exocytosis (39, 40) will be needed to address these questions in acute pituitary slices.

In addition to exocytosis, other Ca^{2+} -dependent functions might also be regulated by rhythmic $[Ca^{2+}]_i$ bursts. One candidate would be hormonal gene expression, which may depend on temporal transitions between silent and $[Ca^{2+}]_i$ spiking periods (41). It should be noted that the bursting pattern comprises two interdependent parameters of timing information: the cycle period (burst plus interburst period) and the duty cycle (percentage of time within a cycle period a cell takes to fire a burst) (20). The dynamic interplay between both parameters could, therefore, provide a subtle code that the endocrine cell could interpret and transduce into the regulation of one or more functions over long time scales.

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