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Calcium Dynamics and Resting Transcriptional Activity Regulates Prolactin Gene Expression

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Research on the regulation of hormone gene expression by calcium signaling is hampered by the difficulty of monitoring both parameters within the same individual, living cells. Here we achieved concurrent, dynamic measurements of both intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and prolactin (PRL) gene promoter activity in single, living pituitary cells. Cells were transfected with the luciferase reporter gene under control of the PRL promoter and subjected to bioluminescence and fluorescence imaging before and after presentation of TSH-releasing hormone (TRH), a prototypic regulator of PRL secretion and gene expression that induces a transient Ca^{2+} release , followed by sustained Ca^{2+} influx. We found that cells displaying specific photonic emissions (*i.e.* mammotropes) showed heterogeneous calcium and transcriptional responses to TRH. Transcriptionally responsive cells always exhibited a TRH-induced $[Ca^{2+}]_i$ increase. In addition, transcriptional responses were related to the rate of Ca^{2+} entry but not Ca^{2+} release. Finally, cells lacking transcriptional responses (but showing $[Ca^{2+}]_i$ rises) exhibited larger levels of resting PRL promoter activity than transcriptionally responsive cells. Thus, our results suggest that the sustained entry of Ca^{2+} induced by TRH (but not the Ca^{2+} release) regulates transcriptional responsiveness. Superimposed on this regulation, the previous, resting PRL promoter activity also controls transcriptional responses. (*Endocrinology* 143: 3548–3554, 2002)

ACTIVATION OF endocrine cells by neurotransmitters and/or extracellular messengers triggers exocytosis of the stored hormone and stimulates hormone gene transcription and biosynthesis (1). This latter process refills the secretory pools and modulates long-term changes in secretory activity. The role of cytosolic calcium in stimulus-secretion coupling has been extensively documented, and single-cell measurements of $[Ca^{2+}]_i$ and exocytosis have provided important clues in this regard (2, 3). In addition, it is becoming increasingly clear that the calcium signal also plays a pivotal role in the control of hormone gene transcription (4, 5). In support of this view, extracellular Ca^{2+} removal and/or Ca^{2+} channel antagonists inhibited hormone gene expression in a variety of tissues and cell types, including prolactin (PRL)-producing cells (mammotropes) (6, 7). Furthermore, several transcription factors involved in control of hormone gene expression are regulated directly (8) or indirectly (9, 10) by calcium signals. In light of the complexity of the role of Ca^{2+} in endocrine cell function, the ability to perform single-cell measurements of calcium dynamics and gene expression in individual cells could provide important new clues (11, 12) on the control of endocrine cell function, especially in those tissues like the anterior pituitary that contain a high degree of cell and functional heterogeneity.

Recently, we have developed a novel approach for monitoring hormone gene expression in individual, living cells (13). This methodology is based on the real-time monitoring of specific photonic emissions from individual cells transfected with the luciferase structural sequence under the control of the PRL gene promoter. We have shown previously that PRL promoter-driven photonic emissions from transfected cells was specific for mammotropes and reflected endogenous activity of the PRL gene (14). In addition, we have characterized the dynamics and demographics of transcriptional responses of mammotropes to TSH-releasing hormone (TRH), a prototypic stimulator of PRL gene expression (15). TRH increases PRL secretion and gene expression, and both processes are mediated by intracellular calcium (16–18). The exact role of calcium in mammotrope function appears to be quite complex. Interestingly, it has been shown that TRH evokes a two-phases increase of $[Ca^{2+}]_i$ and PRL secretion (19, 20). The first phase corresponds to a large but transient (<1 min) $[Ca^{2+}]_i$ rise caused by release of Ca^{2+} from inositol 1,4,5-triphosphate-dependent intracellular stores (*i.e.* the endoplasmic reticulum). The second phase consists of a lower but more sustained $[Ca^{2+}]_i$ increase essentially caused by enhanced Ca^{2+} influx through both voltage-dependent Ca^{2+} channels activated upon enhanced electric activity and store-operated Ca^{2+} entry (19, 20). Thus, information to date suggests that calcium is important in PRL gene expression as well as in cell responsiveness to TRH. In light of these observations, it appears that the combined study of PRL gene expression and calcium in individual cells would provide an excellent model system for beginning to elucidate the processes underlying endocrine cell responsiveness.

Toward this end in the following study, we used a novel strategy based on the concurrent measurement of intracellular Ca^{2+} and PRL reporter expression in the same single, living mammotropes. Our results indicate that the resting level of transcriptional activity and Ca^{2+} entry, but not Ca^{2+}

Abbreviations: FBS, Fetal bovine serum; PRL, prolactin; rPRL-LUC, plasmid that contains the luciferase structural sequence under control of the rat PRL gene promoter; TRH, TSH-releasing hormone; VGCC, voltage-gated Ca^{2+} channel.

release from intracellular stores, regulates transcriptional responses to TRH stimulation.

Materials and Methods

Cell dispersion and microinjection

Primiparous, lactating female (d 6–10 postpartum) rats (Sprague Dawley Harlan, Madison, WI) were maintained on a 12-h light, 12-h dark photo regimen and provided with food and water *ad libitum*. Rats were killed by decapitation and their anterior pituitary glands were removed and dispersed with trypsin as reported previously (14, 15). Monodispersed cells were plated on coverslips coated with poly-L-lysine and photoengraved previously with a numbered/lettered grid pattern (21) to enable cell reidentification of microinjected cells. After 48 h in culture, cells within a particular grid were microinjected with a reporter plasmid (rPRL-LUC, 0.2 μg/μl in 10 mM PBS) in which 2.5 kb of the 5′-flanking region (−2430 to +39) of the rat PRL gene were placed upstream of the coding sequence for firefly luciferase. Cell microinjection was performed and controlled as described previously (14) to ensure the delivery of the same amount of plasmid among cells. After microinjection, cells were washed twice and cultured for 24 or 48 h in phenol red-free DMEM (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10 mM HEPES, 10% fetal bovine serum (FBS), 0.1% BSA, and antibiotics.

Concurrent monitoring of calcium dynamics and PRL gene expression

For concurrent monitoring of Ca^{2+} dynamics and promoter activity on the same individual living cells, we adapted a conventional digital imaging system. For this end, a VIM photonic camera (Hamamatsu Photonics, Bridgewater, NJ) was mounted under the stage of an epifluorescence microscope (Zeiss Axiovert 135 TV, Jena, Germany), which also was equipped with a cooled CCD camera (Hamamatsu Photonics). Excitation light was carried by an optical fiber. A motor-controlled switch allowed capture of either bioluminescence or fluorescence manually or by *ad hoc* software developed by Universal Imaging (West Chester, PA).

The experiments were carried out as follows: First, we measured photonic emissions as described previously (15). In brief, transfected cells were incubated in phenol-free DMEM supplemented with 0.1% BSA, 10% FBS, 10 mM HEPES, and antibiotics for 24 or 48 h. Four hours before measurements of reporter activity, cells were incubated with the same medium containing 0.1 mM luciferin (Sigma, St. Louis, MO). Then, coverslips were assembled in Sykes-Moore chambers and filled with culture medium of the same composition as before except that it was devoid of BSA and bicarbonate and contained FBS and luciferin. The assembled chamber was then placed on the heated (37 C) stage of the microscope, and transfected cells were reidentified according to their position on the numbered/lettered grid. A bright-field image of the microscope field was then captured for reference purposes. Photonic emissions from individual cells were collected in 30-min bins for 4 h to monitor resting transcriptional activity, and the images obtained were stored for further analysis. Then, cells were perifused with culture medium containing fura-2/AM (4 μM) and incubated for 45 min. Once the cells had been loaded with fura-2, the same microscopic field used for photon counting was subjected to $[Ca^{2+}]_i$ measurements before and after addition of TRH (1 μM). For this end, the cells were epiilluminated alternately at 340 and 380 nm (100 msec every 5 sec) using an alternating filter wheel and a fiber light scrambler (Technica Video, Woods Hole, MA). Light emitted above 520 nm was recorded by the cooled CCD camera. The images were stored and analyzed using the Metafluor software from Universal Imaging. Pixel-by-pixel ratios of the 340/380-nm fluorescences were produced and converted into $[Ca^{2+}]_i$ values by comparison with fura-2 standards (22).

Once $[Ca^{2+}]_i$ measurements were performed, cells were perifused again with the same luciferin-containing medium used earlier for photonic emission measurements except that TRH (1 μM) was added. The effect of this treatment on transcriptional activity was recorded by monitoring photonic emissions in 30-min bins for 14 h, and the images were stored for analysis. The relative amount of light emitted by individual mammotropes was quantified by superimposing the images of activated pixels (corresponding to amplified photonic signals) on a frame over the perimeter of the corresponding cell in the bright-field image. The same frame was then superimposed randomly on at least three adjacent areas in the same field devoid of cells to obtain a background value that was subtracted from total counts to calculate specific photonic emissions. Comparisons between treatment groups were made using a two-tailed, *t* test. Results are expressed as mean ± SEM.

Results

Figure 1 illustrates the procedure for concurrent measurement of gene expression and $[Ca^{2+}]_i$ in the same single cells.

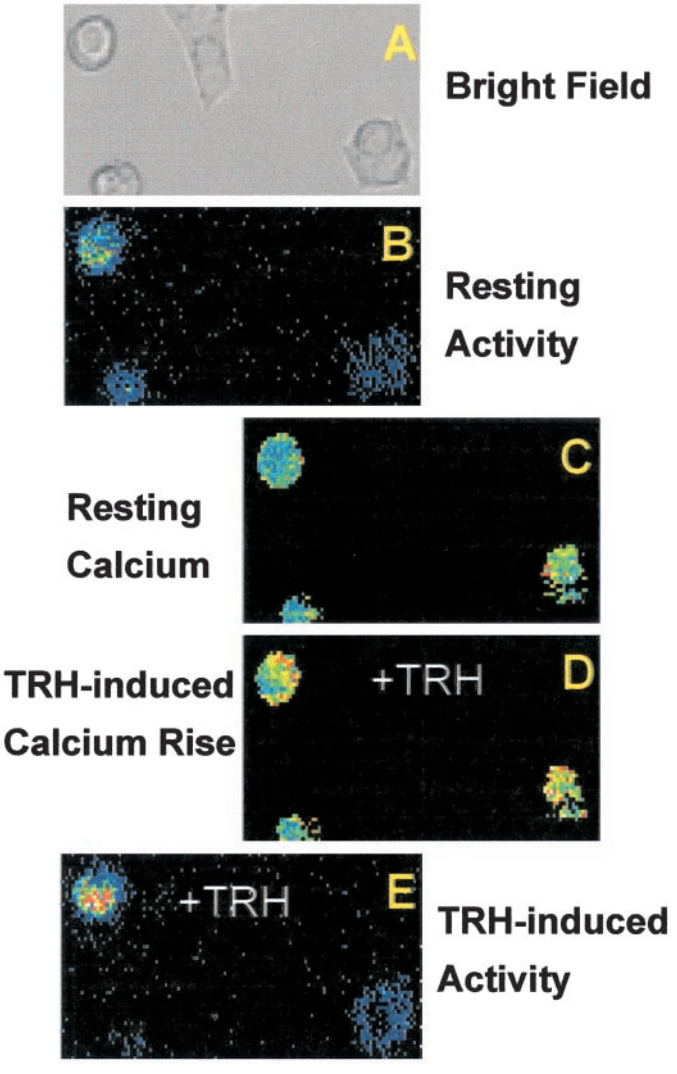

FIG. 1. Imaging of calcium dynamics and PRL gene expression in the same individual primary mammotropes. Anterior pituitary cells were processed as stated in *Materials and Methods* and used for concurrent measurements of fluorescence and bioluminescence. Shown here is a representative cell field in which a bright-field image was captured with the cooled CCD camera (A). Cells were exposed to luciferin and photonic emissions representative of resting transcriptional activity was captured in 30-min bins (B) during 4 h. Then, cells were loaded with fura-2 and $[Ca^{2+}]_i$ was monitored before (C) and after (D) TRH treatment. Finally, the effects of such treatment on transcriptional activity (E) were monitored in 30-min bins for up to 14 h. Warmer colors in the pseudocolor images represent higher values of $[Ca^{2+}]_i$ and PRL gene transcriptional activity.

Cells were microinjected with a reporter plasmid containing luciferase under control of the PRL gene promoter, and 24 h later they were incubated in luciferin-containing medium and subjected to photon counting imaging. After capture of a bright-field image with the photon counting camera (A), photonic emissions reflective of PRL gene expression were recorded in 30-min bins (B) during 4 h. These measurements are representative of resting reporter activity. Then, the cells were loaded with fura-2, and cytosolic Ca^{2+} dynamics was monitored before (C) and after (D) stimulation with TRH. Finally, photonic emissions on stimulation with TRH were recorded for a further 14-h period (E). Only cells displaying photonic emissions above background that emitted light throughout the entire measurement period (heretofore referred as to transfected cells), and loaded fura-2 were included for analysis. This procedure allows recording and comparison of the changes in $[Ca^{2+}]_i$ and PRL gene expression in the same single cells in response to the challenge with TRH.

Representative, single-cell recordings of $[Ca^{2+}]_i$ (*left*) and PRL gene expression (*right*) in the same individual cells are shown in Fig. 2 to illustrate the three different behaviors found regarding responsiveness to TRH. We considered cells responsive to TRH in terms of calcium (23) if transfected cells showed a TRH-induced $[Ca^{2+}]_i$ increase larger than 50 nM (Fig. 2, *left panels*, all cells except red trace in A). We considered transcriptionally responsive cells as those transfected cells showing a clear increase in the rate of photonic emissions after TRH stimulation to reach a value of activity larger than prestimulatory activity (Fig. 2, *right panels*, *blue cells*). Although seemingly arbitrary such decisions were clear-cut as reported previously (15). Transfected cells that did not respond to TRH with an increase of $[Ca^{2+}]_i$ (A, *red cell*) did not increase PRL gene expression either (B, *red cell*). In the cells that responded to TRH with an increase of $[Ca^{2+}]_i$ two different behaviors were found regarding PRL gene expression. First, some cells responded to TRH with an increase in photonic emissions (B, D, and F, *blue cells*), whereas others did not (D and F, *red cells*).

Figure 3 shows the averaged values of $[Ca^{2+}]_i$ (*left*) and their corresponding transcriptional profiles (*right*) of 62 individual cells studied in 9 independent experiments. Cells have been grouped into three different pools: cells that did not respond to TRH with a $[Ca^{2+}]_i$ increase (*upper panels*); cells that responded to TRH with an increase of PRL gene expression (*middle panels*); and cells that were not included in any of the above groups, *i.e.* cells in which TRH induced $[Ca^{2+}]_i$ rise but did not show increased PRL gene expression (*lower panels*). Group i included 19% of the cells and was absolutely homogeneous, *i.e.* all the cells failing to respond to TRH with an increase of $[Ca^{2+}]_i$ also failed to exhibit an increase in gene expression. Group ii included 35% of the cells. In this group the averaged $[Ca^{2+}]_i$ increase at the Ca^{2+} peak was 360 ± 45 nM (n = 22) and the transcriptional

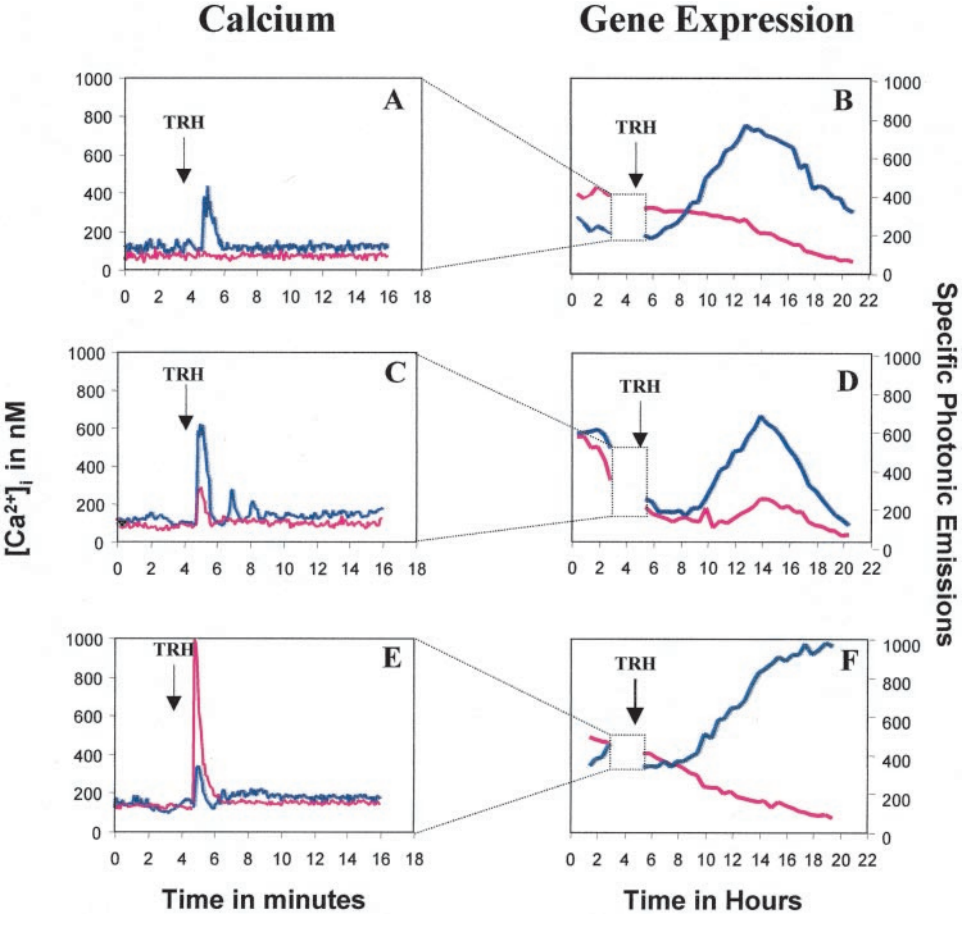

FIG. 2. Calcium and transcriptional responses to TRH are extremely heterogeneous. Each *panel* in the *left column* shows short-term (minutes) fluorescence profiles for two individual cells (*red* and *blue*) present in the same microscopic fields. The long-term (hours) bioluminescence profiles for the same cells are presented to the right (*i.e. blue trace* in A corresponds to the *blue trace* in B). Notice that traces in *left panels* corresponding to $[Ca^{2+}]_i$ measurements were performed during the TRH presentation period of the *right panels*, respectively. Shown are representative examples of 62 individual mammotropes studied in nine independent experiments.

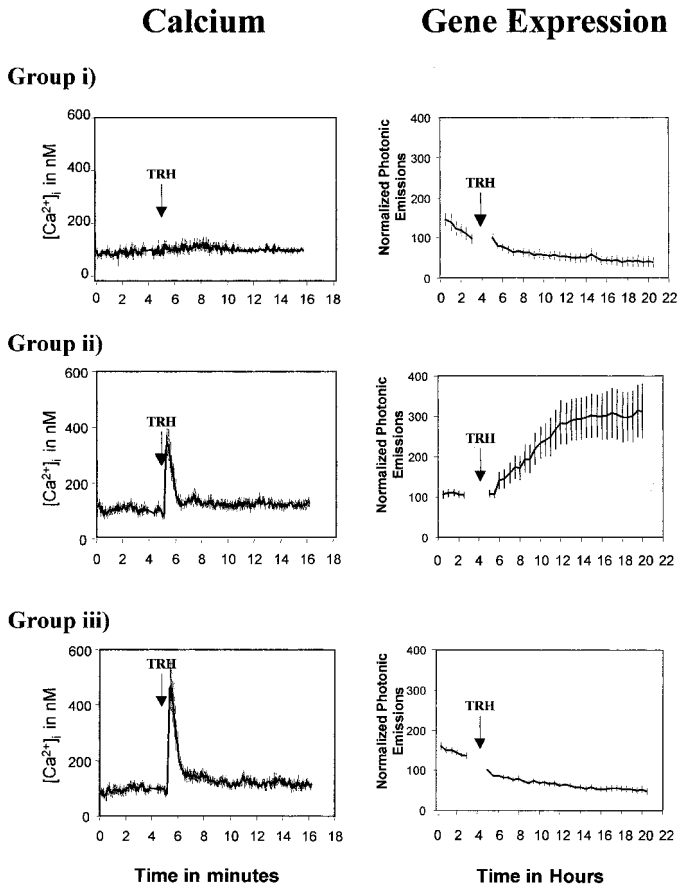

FIG. 3. Averaged $[Ca^{2+}]_i$ increases induced by TRH in both transcriptional responders and nonresponders. Mammotropes were pooled into three different subpopulations: cells that failed to exhibit a calcium response to TRH (n = 12, *top panels*; cells that exhibited a transcriptional response (n = 22, *middle panels*); and cells not included in the above-mentioned groups, *i.e.* cells exhibiting a calcium response but not a transcriptional one (n = 28, *bottom panels*). Cells responsive to TRH in terms of calcium were those transfected cells showing a TRH-induced $[Ca^{2+}]_i$ increase larger than 50 nM after TRH. Transcriptionally responsive cells were considered all those transfected cells showing a clear increase of photonic emissions after TRH relative to the resting level before TRH presentation. The calcium (*left panels*) and transcriptional (*right panels*) profiles for these cell subpopulations were averaged (±SEM) and plotted. Only cells that loaded fura-2 and emitted light above background throughout the entire measurement period were considered for analysis. Data are representative of 62 individual mammotropes studied in nine independent experiments.

activity increased, on average, more than three times within 8–10 h of TRH challenge. Group iii included 44% of the cells. In this group transcriptional activity was not increased or even tended to decrease with time (Fig. 2F, *red cell*), even though the $[Ca^{2+}]_i$ rise induced by TRH was similar to the one found in group ii of cells (430 ± 60 nM, n = 28). These results suggest that the increase of $[Ca^{2+}]_i$ is necessary but not sufficient to trigger the TRH-induced transcriptional response.

Next, we examined the influence of the magnitude of the $[Ca^{2+}]_i$ rises on the transcriptional responses. In most but not all calcium responsive cells, TRH induced the previously identified pattern (19, 20) of $[Ca^{2+}]_i$ change that consisted of a large but transient $[Ca^{2+}]_i$ increase (termed first phase), followed by a lower but more sustained $[Ca^{2+}]_i$ elevation (termed second phase). The relative sizes of both $[Ca^{2+}]_i$ phase changes varied greatly among cells. Figure 4 shows the $[Ca^{2+}]_i$ profiles (A) and the corresponding transcriptional recordings (B) of two individual, representative cells. Interestingly, we noticed that cells showing larger values of second-phase $[Ca^{2+}]_i$ increase tended to show larger transcriptional responses (Fig. 4B). To analyze the relative contribution of each phase to activation of the PRL promoter, we plotted the size of the large, transient TRH-induced $[Ca^{2+}]_i$ peak (first phase) against the corresponding transcriptional response quantified as the maximum increase of photonic emissions (Fig. 4C) and found no correlation ($r = -0.15$, $P > 0.05$) between both parameters. However, we found a positive, significant correlation ($r = 0.56$, $P < 0.01$) between the size of the lower, sustained $[Ca^{2+}]_i$ increase (second phase) and the stimulus-induced transcriptional response (Fig. 4D). Thus, these data suggest that the extent of PRL gene promoter activation induced by TRH relates to the entry of Ca^{2+} during the second phase of the TRH effects rather than to the transient release of Ca^{2+} induced by TRH (first phase).

The above-mentioned results, however, do not explain why the cell population (Fig. 3, group iii of cells) showing a similar Ca^{2+} response than transcriptionally responsive cells (Fig. 3, group ii of cells) lacked increased PRL promoter activity after TRH presentation. We have reported recently that the resting level of PRL gene expression may influence the ability of individual cells to exhibit a transcriptional response. To investigate whether this is the case here, we plotted the increase of photonic emissions induced by TRH against the previous, resting activity for all cells showing transcriptional responses. Figure 5A shows the negative correlation found between the resting transcriptional activity and transcriptional responses ($r = -0.70$, $P < 0.001$). Moreover, we found that resting PRL promoter activity of cells lacking transcriptional responses (but showing TRH-induced $[Ca^{2+}]_i$ increases) was about 4 times larger ($P < 0.05$) than resting promoter activity of transcriptional responders. Thus, these data indicate that resting transcriptional activity influences transcriptional responses to TRH and may explain the lack of transcriptional responsiveness of group iii of cells.

Discussion

Imaging of photonic emissions from transfected mammotropes is an excellent procedure for dynamic monitoring of transcription from single, primary mammotropes for several reasons (14, 15). First, expression of the rPRL-LUC plasmid that contains the luciferase structural sequence under control of the rat PRL gene promoter is specific for mammotropes. This was revealed by our earlier validation experiments (14) showing that all cells displaying specific photonic emissions were also found by immunocytochemistry to store PRL by *in situ* hybridization to contain PRL mRNA or by reverse hemolytic plaque assay to release PRL. Second, experiments performed with agonists and antagonists of PRL gene expression indicated that luciferase activity reflected endogenous PRL gene activity (14). Finally, the noninvasive nature

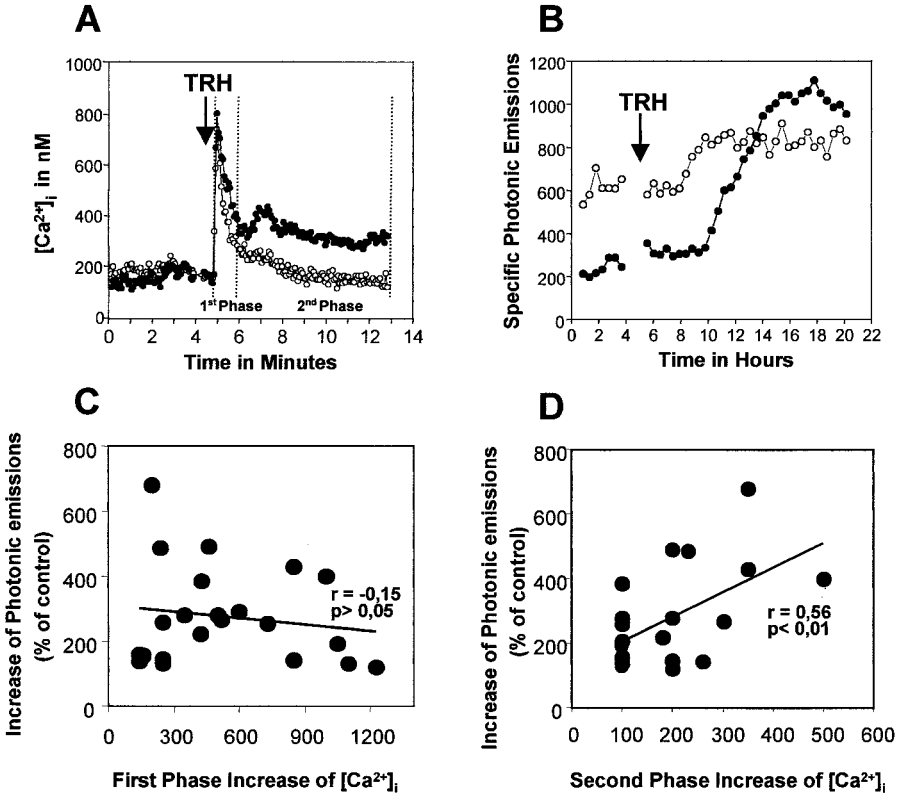

FIG. 4. Relationship between the TRH-induced $[Ca^{2+}]_i$ rises and transcriptional responses. The profiles of $[Ca^{2+}]_i$ (A) and the corresponding transcriptional recordings (B) of two individual, representative cells are shown. Notice that the effects of TRH on $[Ca^{2+}]_i$ consisted of a large, transient $[Ca^{2+}]_i$ increase (<1 min), termed first phase, followed by a lower but more sustained $[Ca^{2+}]_i$ increase, termed second phase. Cells displaying larger second-phase $[Ca^{2+}]_i$ increases (A, *full circles trace*) tended to show larger transcriptional responses (B) than cells having lower second phase $[Ca^{2+}]_i$ increase (A, *open circles trace*). C, Lack of correlation between the first phase of TRH-induced $[Ca^{2+}]_i$ increase (maximum $[Ca^{2+}]_i$ value during the first minute) and the transcriptional response measured as the maximum increase of photonic emissions after TRH presentation ($r = -0.15$, $P > 0.05$, $n = 22$). D, Positive correlation between the second phase of the TRH-induced $[Ca^{2+}]_i$ increase (averaged $[Ca^{2+}]_i$ value from 1 min after TRH presentation to the end of the trace) and the transcriptional response ($r = 0.56$, $P < 0.01$, $n = 22$). These data were taken from the 22 transcriptionally responsive cells studied (group ii of cells in Fig. 3).

of this procedure and the short half-life of the reporter enzyme enabled the actual monitoring of transcriptional activity (24). The data presented herein demonstrate the feasibility of combining this novel technology with fluorescence measurements of $[Ca^{2+}]_i$ as shown by the following observations: 1) After more than 4 h of photonic emission measurements, cells loaded fura-2 normally and responded to TRH with the typical rise of $[Ca^{2+}]_i$; 2) the fraction of calcium responders was similar to that found in cells not transfected or subjected to long-term monitoring of transcriptional activity (23); and 3) 35% of the cells exhibited clear transcriptional responses, and this number compares well with the relative abundance of transcriptional responders found in cells not loaded with fura-2 (15), thus indicating strongly that the fura-2 loading does not have deleterious effects on transcriptional activity and/or responsiveness. Taken together, these data demonstrate the feasibility of concurrent monitoring of $[Ca^{2+}]_i$ and transcriptional dynamics in single, living mammotropes.

It is well known that not all mammotropes respond to TRH with an increase of $[Ca^{2+}]_i$ and a rise of PRL secretion (14). Accordingly, it should be expected that some cells fail to show increased PRL gene expression after TRH presentation. We found that cells showing no $[Ca^{2+}]_i$ rise after TRH did not exhibit any increase of transcriptional activity. However, the reverse did not apply and not all cells showing TRH-induced $[Ca^{2+}]_i$ rise were able to mount the corresponding transcriptional response. Therefore, the $[Ca^{2+}]_i$ rise appears necessary but not sufficient for stimulation of transcriptional activity. In addition, we have shown that the size of the transcriptional response is positively correlated to the size of the second-phase TRH-induced $[Ca^{2+}]_i$ increase but not to the first one. It is well established that the first phase is attributed to Ca^{2+} release from intracellular stores (*i.e.* the endoplasmic reticulum), whereas the second one corresponds to a sustained Ca^{2+} influx mostly through voltage-gated Ca^{2+} channels (VGCCs) (19). Therefore, our results indicate that the extent of transcriptional response to TRH is driven by the sustained entry of calcium induced by the secretagogue rather than by the transient release of Ca^{2+}. It is well established that TRH-induced Ca^{2+} entry on activation of L-type VGCCs is secondary to enhanced electric activity achieved by inhibition of an inward rectifying K^+ current of the h-erg type (25). Previous reports in cell populations have established that inhibition of Ca^{2+} entry through L-type VGCCs decreased PRL gene expression, whereas stimulation of Ca^{2+} through the same channels increased PRL gene expression (6, 7).

Taken together, our results indicate that the effects of TRH on activation of the PRL promoter activity are directly related to the rate of Ca^{2+} entry through L-type VGCCs and thus to inhibition of the herg-type K^+ current. It is becoming evident that the source of Ca^{2+}; therefore the Ca^{2+} rise amplitude and duration evokes differential activation of transcription factors. For example, nuclear factor-$\kappa\beta$ and c-Jun N-terminal kinase transcription factors are selectively activated by a large transient Ca^{2+} rise, whereas nuclear factor of activated T cells transcription factor is activated by a low, sustained Ca^{2+} plateau (11). Our results indicate that PRL gene expression is sensitive to a low sustained Ca^{2+} entry through L-type VGCCs but not to a transient Ca^{2+} release from internal stores. An important question is how the Ca^{2+} signal

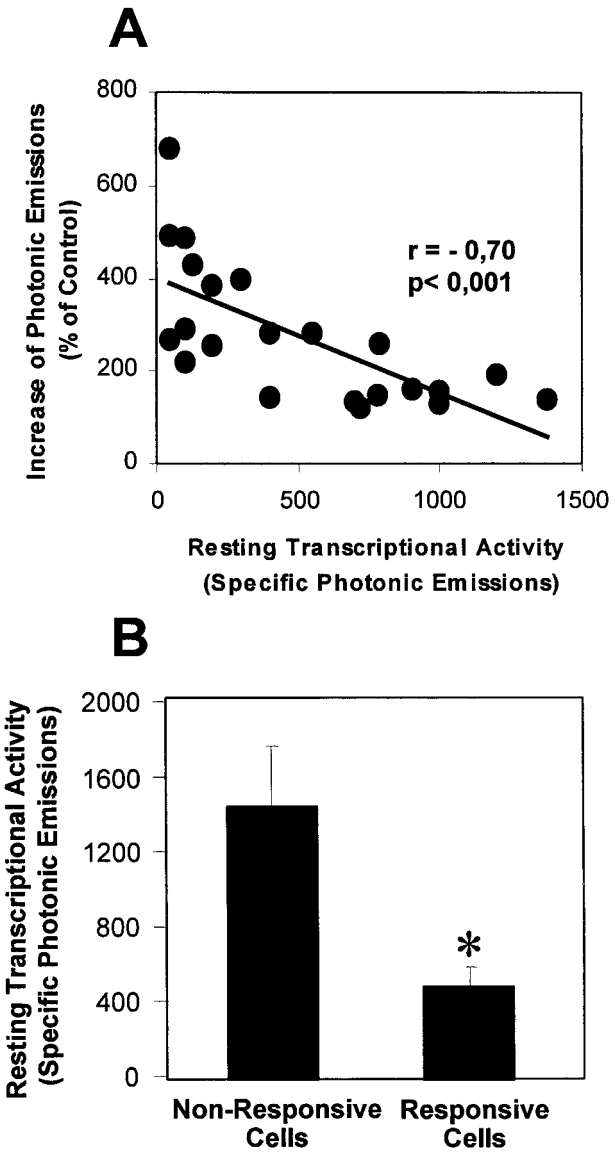

FIG. 5. Resting transcriptional activity regulates transcriptional responsiveness to TRH. A, Negative correlation between resting transcriptional activity and transcriptional responses for cells exhibiting increases in photonic emissions after TRH presentation ($r = -0.7, P < 0.001$, n = 22). B, Basal PRL promoter activity (photonic emission values) for cells, which subsequently proved to be TRH responsive (Responsive cells, n = 22) or nonresponsive (nonresponsive cells, n = 28) in terms of gene expression are compared, *, $P < 0.05$.

is brought from the plasma membrane Ca^{2+} channel to the nucleus. Interestingly, Dolmetsch *et al.* (26) have reported recently a signaling to the nucleus by an L-type Ca^{2+} channel-calmodulin complex through the MAPK pathway. Wang and Maurer (27) have recently provided evidence that TRH induces a sustained activation of MAPK to stimulate the PRL promoter. Importantly, activation of this MAPK pathway was inhibited by blocking of L-type VGCCs (27). Thus, our results are consistent with the possibility that sustained entry of Ca^{2+} induced by TRH could regulate PRL gene expression by means of a Ca^{2+} channel-calmodulin complex and the MAPK signaling pathway.

The above discussed results cannot explain why cells exhibiting a similar TRH-induced $[Ca^{2+}]_i$ rise fail to mount a transcriptional response (Fig. 3, group iii). One possible explanation is that downstream signaling elements involved in promoter activity are down-regulated in the transcriptionally reluctant cells. If this were the case, then it would be expected than the resting level of PRL gene promoter activity could be likewise down-regulated. However, we found that, on the contrary, resting PRL promoter activity was larger in cells not responsive transcriptionally to TRH. We had previously reported that resting transcriptional activity may influence the ability of TRH to increase PRL gene promoter activity. However, no $[Ca^{2+}]_i$ measurements were performed in that study, and, therefore, it was not possible to establish whether lack of transcriptional responsiveness was due to lack of TRH receptors. Here we found that cells lacking transcriptional responses to TRH (but not TRH receptors) showed a significantly larger resting PRL promoter activity than transcriptionally responsive cells. In addition, we found that the extent of the transcriptional response was inversely related with the previous, resting promoter activity. Thus, our results indicate that a $[Ca^{2+}]_i$ rise appears necessary for elaboration of transcriptional responses to TRH. However, whether the cell is actually going to respond and the extent of the response is dictated by the previous transcriptional status of the cell. We and others (15, 28) have shown that the resting activity of the PRL gene promoter exhibit dramatic fluctuations (pulses) over time. Furthermore, the recent construction of a destabilized luciferase reporter gene (29) has enabled the characterization of transcriptional pulses that oscillate in a noncircadian temporal pattern (30). As a result, the changes of transcriptional responses to TRH and perhaps to other stimuli may depend on the state of the transcriptional cycle and may occur independently of changes in expression of TRH receptors.

Our results pose the question on how the transcriptional status of a given cell, calibrated here as the relative level of resting activity regulates downstream signals derived directly or indirectly from the stimulus-induced $[Ca^{2+}]_i$ rise. One possibility is that transcription factors involved in Ca^{2+} regulation of PRL gene expression are already active (either by phosphorylation and/or translocation to the nucleus) at resting $[Ca^{2+}]_i$. This would explain the higher resting activity and lack of transcriptional responsiveness. If this were the case, then what mechanism would activate the putative transcription factor/s involved? One possibility is a that a previous Ca^{2+} signal developed long before might elicit such activation. In support of this view, we have reported recently that mammotropes undergo dynamic changes in spontaneous $[Ca^{2+}]_i$ oscillations (24). Moreover, at a given point in time, oscillatory activity and hormone gene expression were inversely related (24), indicating the temporal dissociation between the calcium signal and the corresponding transcriptional response. Therefore, we propose that calcium dynamics is involved in transcriptional responsiveness by two mechanisms. First, it plays an obligatory role for stimulus-induced PRL gene expression and second, by modulating resting transcriptional dynamics regulates responsiveness to the incoming calcium signal. Alternatively, transcription factors not driven by calcium signals but required for transcrip-

tional responses may undergo cycles of activation/inactivation driven by an endogenous transcriptional clock that may work as a coincidence detector for calcium-mediated signals. The nature of this putative transcriptional clock remains to be established.

In summary, we have developed a system to enable dual measurements of both bioluminescence and fluorescence on the same individual, living cells and use it to achieve concurrent measurements of both $[Ca^{2+}]_i$ and hormonal gene expression in the same single cells. We have provided evidence indicating that the size of transcriptional responses to TRH are dictated by the sustained entry of calcium into the cell. In addition to this regulation, the resting level of transcriptional activity that oscillates in a noncircadian temporal pattern (30) may superimpose a timing control on transcriptional responses.

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