Endocrine, *vol. 7, no. 3, 343–349, December 1997* 0969-711X/97/7:343-349/$9.75

Relationships Between Dopamine-Induced Changes in Cytosolic Free Calcium Concentration ($[Ca^{2+}]_i$) and Rate of Prolactin Secretion

Elevated $[Ca^{2+}]_i$ Does Not Indicate Prolactin Release

Annisa Chang and Seon H. Shin

Department of Physiology, Queen's University, Kingston, Ontario, Canada

This study was undertaken to investigate the relationship between dopamine (DA) induced changes in the cytosolic calcium concentration ($[Ca^{2+}]_i$) and the rate of prolactin secretion using GH_4ZR_7, a rat pituitary cell line, which express only one subtype of D_2 receptor. GH_4ZR_7 cells were loaded with Fluo-3, a fluorescent Ca^{2+} indicator, and then perifused with two different doses of DA (10^{-7} mol/L and 5×10^{-4} mol/L). We monitored changes in $[Ca^{2+}]_i$ and rate of prolactin release simultaneously by attaching a spectrofluorometer to a dynamic perifusion system. DA has stimulatory and inhibitory effect on prolactin secretion in GH_4ZR_7 cells; 10^{-7} mol/L DA slightly increased $[Ca^{2+}]_i$ and stimulated prolactin release, whereas 5×10^{-4} mol/L DA decreased $[Ca^{2+}]_i$ and inhibited prolactin secretion. When the cells were pretreated with pertussis toxin (PTX), 10^{-7} mol/L DA had no significant change in $[Ca^{2+}]_i$ while stimulating prolactin release, and 5×10^{-4} mol/L DA reduced $[Ca^{2+}]_i$ without having any significant effect on the rate of prolactin secretion. The results of this study demonstrate that changes in $[Ca^{2+}]_i$ do not always correlate with the rate of prolactin release from lactotrophs. The dissociation between $[Ca^{2+}]_i$ and prolactin release is somewhat expected considering the diverse role of $[Ca^{2+}]_i$ and post-$[Ca^{2+}]_i$ events, which can change the rate of prolactin release.

Key Words: Dopamine; cytosolic calcium; prolactin; fluo-3; GH_4ZR_7.

Introduction

The synthesis of prolactin and its release from lactotrophs have been extensively studied over the past two decades. The role of dopamine (DA) as the predominating physiological inhibitor in prolactin release *(1–3)*, and at much lower concentrations, a stimulator in prolactin secretion from lactotrophs has been demonstrated both in vitro *(4–10)* and in vivo *(11)*. DA, however, does not act alone; for example, it cannot sustain its inhibitory action without ascorbic acid in the blood *(12)*. Cytosolic free calcium plays a critical role in many signal transduction systems, and it is required for prolactin secretion *(3,13)*. Many independent studies have concluded that DA inhibits calcium current (Ica) *(14–16)*, which in turn alters the cytosolic free calcium concentration ($[Ca^{2+}]_i$) *(7,17–20)*. Nevertheless, the relationship between $[Ca^{2+}]_i$ and the rate of prolactin secretion is still poorly understood. We therefore examined whether the DA-induced changes in the rate of prolactin release are directly correlated with changes in $[Ca^{2+}]_i$ using GH_4ZR_7 cells loaded with Fluo-3, a fluorescent Ca^{2+} indicator.

Received July 8, 1997; Revised September 3, 1997; Accepted September 3, 1997.

Author to whom all correspondence and reprint requests should be addressed: Dr. S. H. Shin, Department of Physiology, Queen's University, Botterell Hall, Kingston, Ontario, Canada K7L 3N6. E-mail: shins@post.queensu.ca

Results

TRH was used as a reference control to ensure that the cells were responding properly to secretagog. With all the fluorescent tracings, it is important to note that the declining baseline of fluorescent intensity does not represent a decreasing $[Ca^{2+}]_i$, but shows leakage of Fluo-3 from the cells. Fluo-3 is a nonratiometric dye. The fluorescent intensities were parallel with those of the control groups, which were not treated with any secretagog (data not shown). We overlaid a baseline into the tracings when we analyzed our data and compared any secretagog-induced changes in the fluorescent intensity to the baseline to compensate for the effect of the declining slope of fluorescent on the results.

The relationship between intensity of fluorescence and $[Ca^{2+}]_i$ was established using the ionomycin and Mn^{2+} technique *(21,22)*. Dulbecco's Modified Eagle's Medium (DMEM) (without bovine serum albumin [BSA]) was used in the calibration procedure, because BSA was found to form hydrophobic bonds with ionomycin, thus reducing

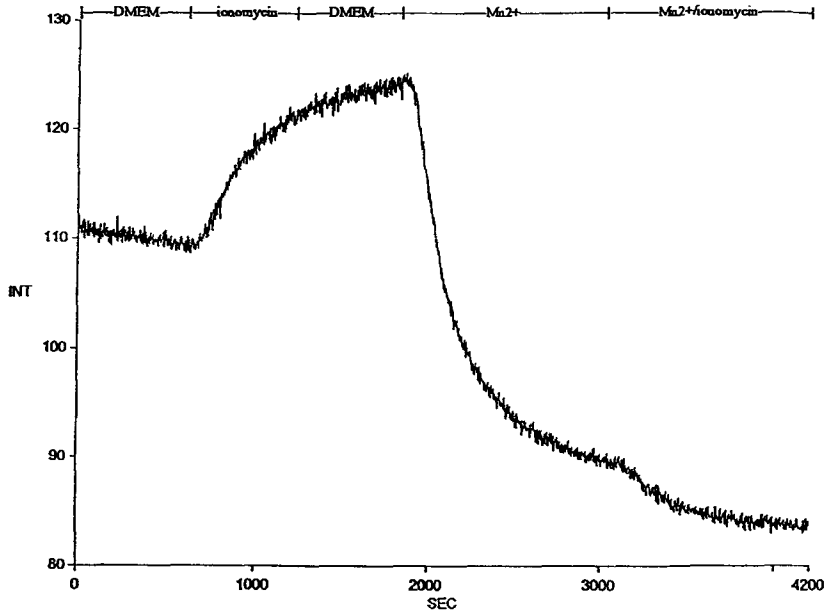

Fig. 1. The relationship between intensity of fluorescence and $[Ca^{2+}]_i$ was established using the ionomycin and Mn^{2+} technique. Control medium (DMEM without BSA), and ionomycin (20×10^{-6} mol/L) were perifused for 10 min each, then control medium, Mn^{2+} (0.2×10^{-3} mol/L), Mn^{2+} (10^{-3} mol/L) plus ionomycin (10^{-6} mol/L) were perifused for 20 min each in sequence. This is a representative tracing out of five independent experiments.

the availability of ionomycin dissolved in DMEM *(22)*. DMEM without BSA was initially perifused through Fluo-3-loaded cells until a calcium fluorescence baseline was established, followed by the perifusion of 10 μmol/L ionomycin in DMEM without BSA. Figure 1 shows the calibration curve. The optical density increased from 109 optical density units (OD) to 124 OD, and the level was sustained (Fig. 1). When the calcium complex of extracellular Fluo-3 was quenched with 0.2 mmol/L Mn^{2+} in DMEM without BSA, the optical density of light emission decreased drastically from 124 OD to 90 OD (Fig. 1). The optical density dropped even further (from 90 OD to 85 OD) when the intracellular calcium–Fluo-3 complex was quenched with 1 mmol/L Mn^{2+} plus 10 mmol/L ionomycin in DMEM without BSA (Fig. 1). $[Ca^{2+}]_i$ was calculated according to Tsien et al. *(23)*, and the value was 106.7 n*M*.

In both the naive (nonpertussis-toxin [PTX] treated) and the PTX-treated GH_4ZR_7 cells, perifusion of TRH (10^{-6} mol/L) triggered a burst of $[Ca^{2+}]_i$ and a significant increase of prolactin release as expected (Figs. 2 and 3). The peak fluorescent intensity induced by TRH in the naive cells reached, on an average, two units of fluorescent intensity above the baseline level ($p < 0.05$; $n = 3$), and the peak percentage change in prolactin concentration reached 263 ± 35% (mean ± SEM) within the first 7 min of TRH perifusion (Fig. 2). Similarly, the peak fluorescent intensity induced by TRH in the PTX-treated cells reached about 3 U of fluorescent intensity above the baseline level ($p < 0.05$; $n = 3$), and the peak percentage change in prolactin concentration reached 277 ± 28% within the first 7 min of TRH

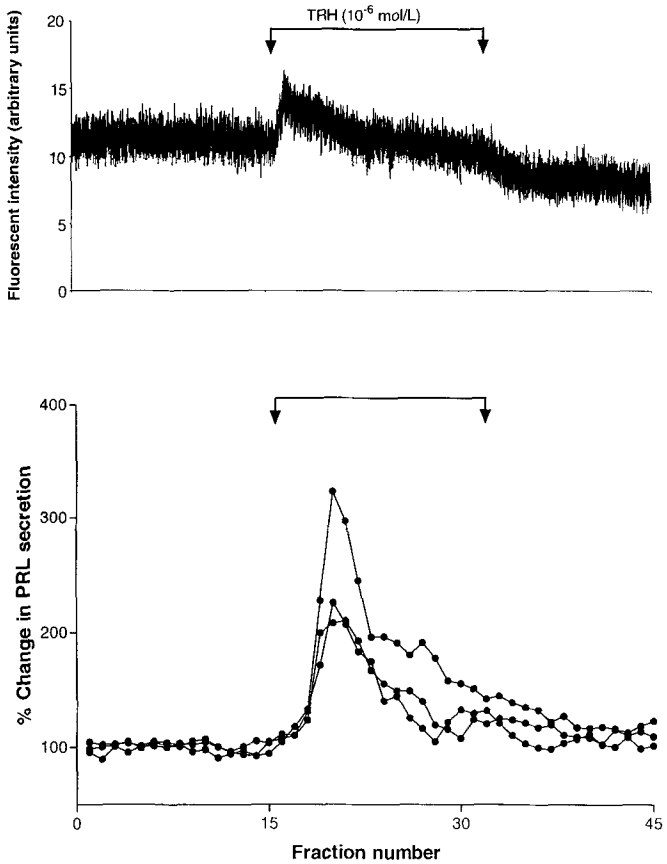

Fig. 2. The effects of TRH (10^{-6} mol/L) on $[Ca^{2+}]_i$ (upper panel) and prolactin release (lower panel) in Fluo-3-loaded GH_4ZR_7 cells. Control medium (DMEM-BSA) (fractions #1–15), TRH (10^{-6} mol/L) (fractions #16–30), and control medium (fractions #31–45) were perifused through the cuvet. Perifusate was collected at the rate of 1 fraction/min. One representative spectrofluorometric tracing and three representative experiments of prolactin secretion are shown.

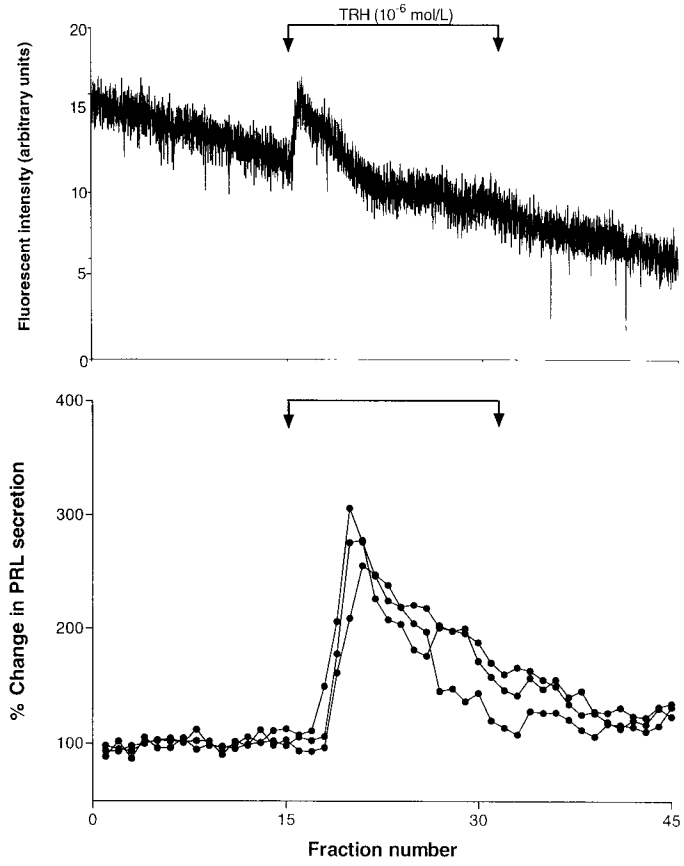

Fig. 3. The effects of TRH (10^{-6} mol/L) on [Ca^{2+}]$_i$ (upper panel) and prolactin release (lower panel) in PTX- (50 ng/mL) pretreated, Fluo-3-loaded GH_4ZR_7 cells. Control medium (DMEM-BSA) (fractions #1–15), TRH (10^{-6} mol/L) (fractions #16–30), and control medium (fractions #31–45) were perifused through the cuvet. Perifusate was collected at the rate of 1 fraction/min. One representative spectrofluorometric tracing and three representative experiments of prolactin secretion are shown.

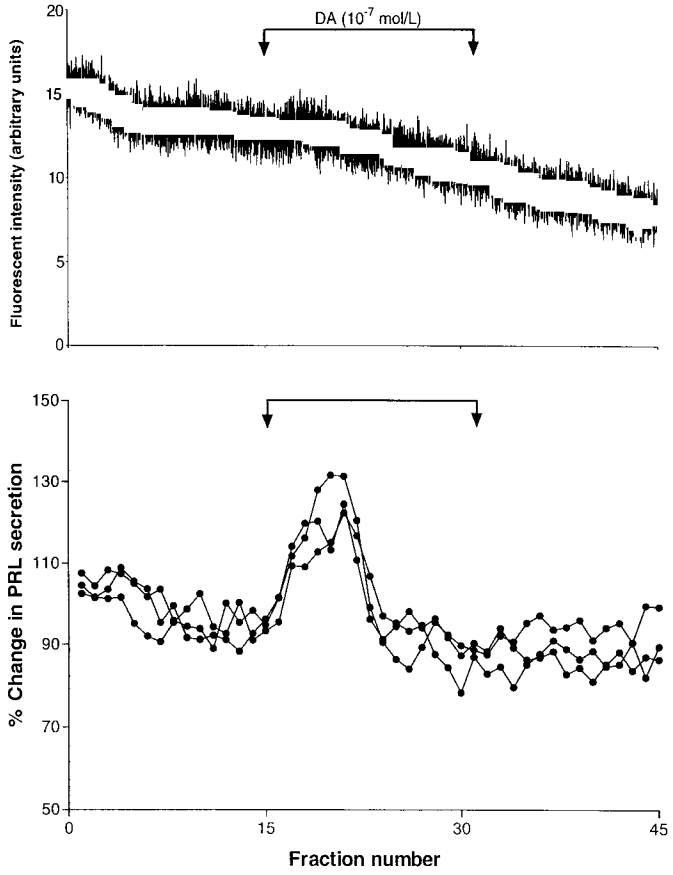

Fig. 4. The effects of DA (10^{-7} mol/L) on [Ca^{2+}]$_i$ (upper panel) and prolactin release (lower panel) in Fluo-3-loaded GH_4ZR_7 cells. Control medium (DMEM-BSA) (fractions #1–15), DA (10^{-7} mol/L) (fractions #16–30), and control medium (fractions #31–45) were perifused through the cuvet. Perifusate was collected at the rate of 1 fraction/min. One representative spectrofluorometric tracing and three representative experiments of prolactin secretion are shown.

perifusion (Fig. 3). The TRH-induced changes in [Ca^{2+}]$_i$ and prolactin secretion in the naive group (Fig. 2) were not significantly different from that of the PTX-treated group (Fig. 3), indicating that PTX had no significant effect on the stimulatory action of TRH on prolactin release. The results also confirmed that [Ca^{2+}]$_i$ is involved in the TRH-induced prolactin release pathway.

When the naive GH_4ZR_7 cells were treated with 10^{-7} mol/L DA, [Ca^{2+}]$_i$ always increased as well as the rate of PRL release. A low dose of DA caused a brief and slight elevation (to about 1.5 U above the basal level) in the fluorescent intensity (Fig. 4). The peak percentage change in prolactin concentration reached 123 ± 11% ($p < 0.05$; $n = 3$) and returned to a baseline concentration during DA perifusion (Fig. 4). On the other hand, a higher concentration of DA (5×10^{-4} mol/L) reduced [Ca^{2+}]$_i$ and decreased the rate of prolactin secretion. In a typical experiment, the fluorescent intensity gradually dropped to about 3 U below the basal level. The prolactin concentration dropped rapidly to its lowest level within the first 7 min of DA treatment and returned quickly to the basal level, whereas depressed [Ca^{2+}]$_i$ was sustained during the entire DA perifusion period (Fig. 5). The percentage change in nadir prolactin concentration reached 77 ± 3% ($p < 0.05$; $n = 3$).

In the PTX-treated cells, a low concentration of DA (10^{-7} mol/L) had no significant effect on [Ca^{2+}]$_i$ ($p > 0.1$); however, it stimulated prolactin release (Fig. 6). Prolactin concentration went up to 133 ± 5% of basal concentration ($p < 0.05$; $n = 3$). In this study, the peak prolactin concentration induced by DA in the PTX-treated group (Fig. 7) was not significantly different from that of the naive group (peak % change: 123 ± 11%) (Fig. 5) indicating that the stimulatory action of DA was not affected by the PTX. A higher concentration of DA (5×10^{-4} mol/L) caused a similar reduction in [Ca^{2+}]$_i$ in the PTX-treated group (Fig. 7) (to about 3 U of fluorescent intensity below basal level) as it did in the naive group (Fig. 5). However, it did not cause any significant change in the rate of prolactin secretion. These observations confirmed that the DA's inhibitory

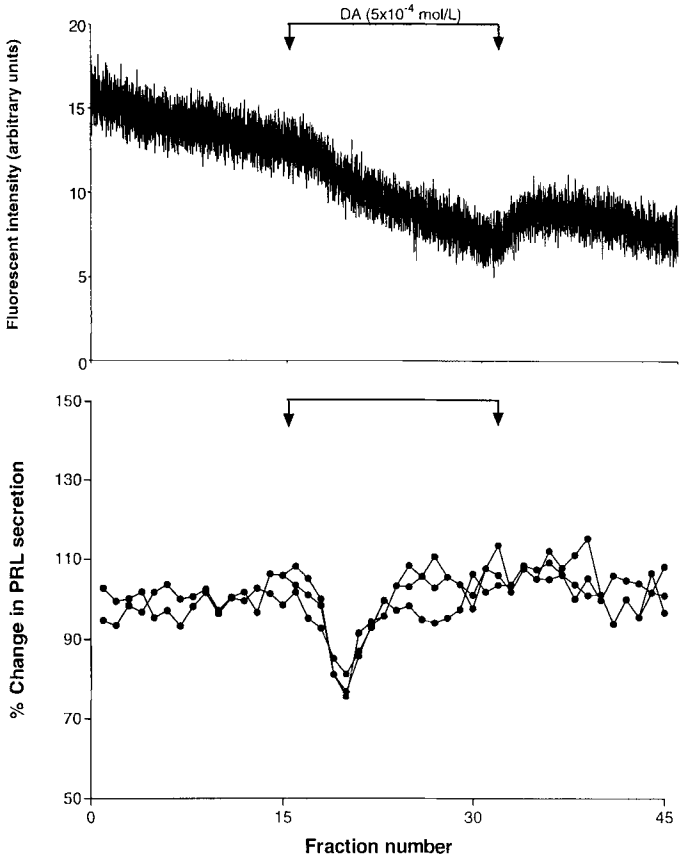

Fig. 5. The effects of DA (5×10^{-4} mol/L) on $[Ca^{2+}]_i$ (upper panel) and prolactin release (lower panel) in Fluo-3-loaded GH_4ZR_7 cells. Control medium (DMEM-BSA) (fractions #1–15), DA (5×10^{-4} mol/L) (fractions #16–30), and control medium (fractions #31–45) were perifused through the cuvet. Perifusate was collected at the rate of 1 fraction/min. One representative spectrofluorometric tracing and three representative experiments of prolactin secretion are shown.

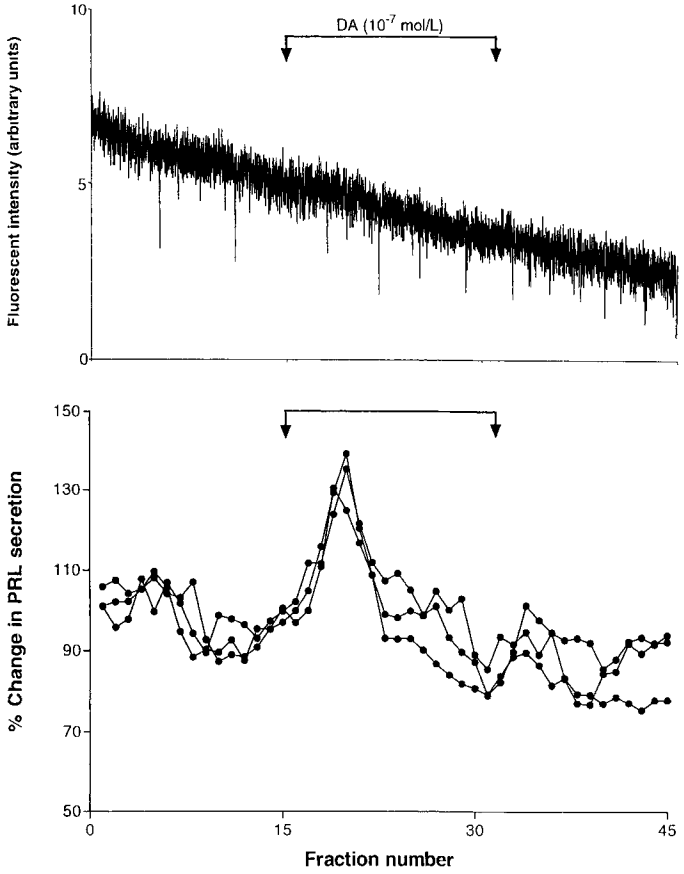

Fig. 6. The effects of DA (10^{-7} mol/L) on $[Ca^{2+}]_i$ (upper panel) and prolactin release (lower panel) in PTX- (50 ng/mL) pretreated, Fluo-3-loaded GH_4ZR_7 cells. Control medium (DMEM-BSA) (fractions #1–15), DA (10^{-7} mol/L) (fractions #16–30), and control medium (fractions #31–45) were perifused through the cuvet. Perifusate was collected at the rate of 1 fraction/min. One representative spectrofluorometric tracing and three representative experiments of prolactin secretion are shown.

action is mediated through G_i-protein and showed that changes in $[Ca^{2+}]_i$ are not always proportional to changes in the rate of prolactin secretion.

Discussion

Most studies concerning changes of $[Ca^{2+}]_i$ were performed with either single cells *(18,19,24)* or suspended cells in a cuvet *(25–27)*. Cell suspension in a cuvet can be used to measure dynamic changes of $[Ca^{2+}]_i$, but the system reveals only the final accumulated concentrations of a released hormone in the medium. Thus, one cannot make any dynamic correlation between alterations in $[Ca^{2+}]_i$ and rates of prolactin release. We applied a technique of spectrofluorometry to a dynamic perifusion system; $[Ca^{2+}]_i$ was measured by Fluo-3 during a perifusion period, but fractions for quantification of prolactin were collected. Therefore, we were able to monitor simultaneously the two important parameters.

Some studies have tried to correlate relationships between changes of $[Ca^{2+}]_i$ and rates of prolactin release *(22,28)*, but they were performed using primary cultured rat pituitary cells. Primary cultured pituitary cells are mixed populations. They include several different lactotroph subtypes *(29)*, and different lactotroph subtypes express different types of DA receptors, which include receptors for the stimulatory and inhibitory actions *(30)*. Different receptors can be activated in different experimental conditions, thus making it difficult to define relationships between $[Ca^{2+}]_i$ and prolactin release. We used cloned GH_4ZR_7 cells, which originate from the rat pituitary tumor cell line, GH_4C_1 · GH_4C_1 cells synthesize and secrete PRL, and express many characteristics of a normal lactotroph, but they do not express DA receptors. GH_4ZR_7 cells evolved after GH_4C_1 cells were transfected with DA D_{2S} receptor-complementary DNA, and they express solely the short isoform of the D_2 receptors *(31)*. Using the cultured GH_4ZR_7 cells is advantageous, because they allowed us to focus on the signaling pathway of a single DA D_2-receptor subtype. On the other hand, GH_4ZR_7 cells are much less sensitive to DA's inhibitory action than primary cultured cells. The difference in sensitivity between the

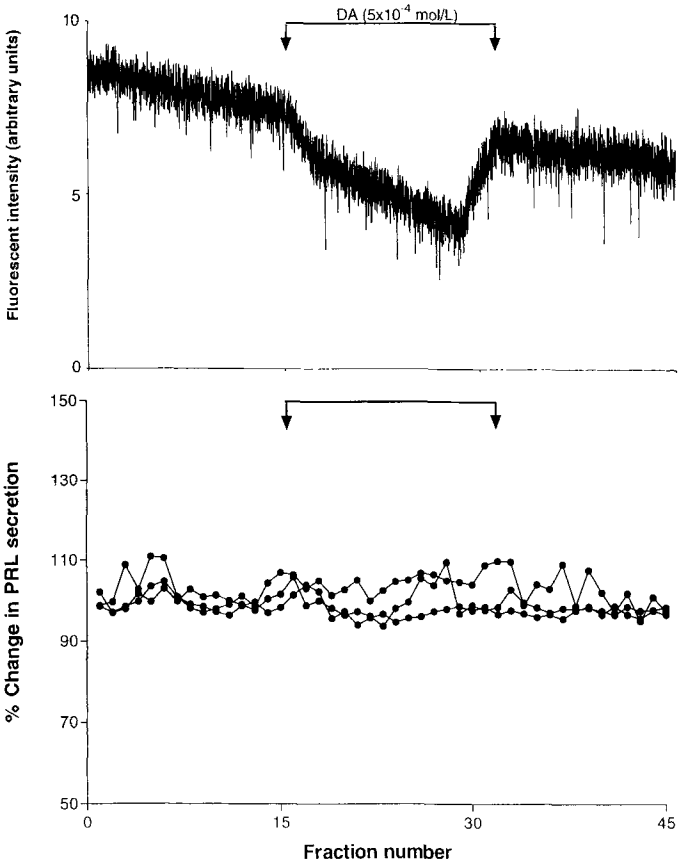

Fig. 7. The effects of DA (5×10^{-4} mol/L) on [Ca^{2+}]$_i$ (upper panel) and prolactin release (lower panel) in PTX- (50 ng/mL) pretreated, Fluo-3-loaded GH_4ZR_7 cells. Control medium (DMEM-BSA) (fractions #1–15), DA (5×10^{-4} mol/L) (fractions #16–30), and control medium (fractions #31–45) were perifused through the cuvet. Perifusate was collected at the rate of 1 fraction/min. One representative spectrofluorometric tracing and three representative experiments of prolactin secretion are shown.

two types of cells can be explained by their not having the same number of active membrane-bound receptors *(10)*. Because of the low sensitivity of GH_4ZR_7 cells to DA, we considered 10^{-7} mol/L DA as a low concentration and 5×10^{-4} mol/L as a high concentration.

[Ca^{2+}]$_i$ plays many important roles in a cell; one of the functions is to stimulate hormone release. There is ample evidence showing that DA elevates and reduces [Ca^{2+}]$_i$ along with prolactin release in a dose-dependent manner *(17)*. A concentration of DA that would stimulate prolactin secretion increased [Ca^{2+}]$_i$, and another concentration that would inhibit prolactin release reduced [Ca^{2+}]$_i$. Thus, it has been generally assumed that the [Ca^{2+}]$_i$ is directly related to the level of prolactin output from the lactotroph cells. However, when we closely examined the relationship between the two parameters, the degree of changes in [Ca^{2+}]$_i$ did not appear to be proportional to the rate of prolactin release from GH_4ZR_7 cells. During a 15-min perifusion period with a low concentration of DA, there was a burst of prolactin release with only a brief and slight increase in the [Ca^{2+}]$_i$ (Fig. 4). On the other hand, when a high dose of DA was perifused through the cells, the rate of prolactin secretion decreased for a relatively short time before returning to the basal level, whereas the level of Ca^{2+} remained at a reduced level (Fig. 5). When PTX-treated cells were perifused with a low dose of DA, prolactin release was stimulated, whereas there was no apparent change in [Ca^{2+}]$_i$ (Fig. 6). Also, when the PTX-treated cells were perifused with the high dose of DA, no significant change in the rate of prolactin release occurred, but the level of [Ca^{2+}]$_i$ was greatly reduced (Fig. 7).

Our results indicate that there is no direct relationship between [Ca^{2+}]$_i$ and the rate of prolactin release. However, the dissociation between [Ca^{2+}]$_i$ and prolactin release should not be surprising considering the diverse actions of [Ca^{2+}]$_i$ in the physiological system. [Ca^{2+}]$_i$ is a link to a series of events in prolactin release, and prolactin secretion can be inhibited or stimulated by activation of other links after the [Ca^{2+}]$_i$ event. In the proposed mechanism, DA binds to the D_{2S} receptors and poses an inhibitory effect on the negatively coupled adenylyl cyclase *(8,32)* via a PTX-sensitive G-protein *(7,10,31,33,34)*. Potassium conductance increases through activation of both the G-protein-regulated *(35,36)* and voltage-dependent potassium channels *(37)*, and the cell membrane hyperpolarizes *(38)*. This leads to inhibition of calcium-dependent action potentials and a reduction in the intracellular calcium concentration *(24)*, and subsequently a decrease in prolactin secretion. The model suggests that the amount of prolactin output depends on the level of [Ca^{2+}]$_i$. Nevertheless, the observed dissociation between prolactin release and changes of [Ca^{2+}]$_i$ illustrates that [Ca^{2+}]$_i$ is a factor, but not the sole, final determinant of prolactin output.

In summary, we have observed dissociation between DA-induced changes in [Ca^{2+}]$_i$ and rates of prolactin secretion in the cloned GH_4ZR_7 lactotroph cells. There is no doubt that an elevated level of [Ca^{2+}]$_i$ is required for prolactin secretion, but the level of [Ca^{2+}]$_i$ is not directly related to the amount of prolactin release.

Materials and Methods

Cell Culture

GH_4ZR_7 cells (kindly provided by H. Elsholtz, University of Toronto) were cultured in a mixture of Ham's Nutrient Mixture F-12 (Connaught Laboratories Ltd., Willowdale, Canada) and DMEM (Gibco Lab., Grand Island, NY) (3:4) supplemented with 2.5% fetal calf serum, 15% horse serum (Gibco Lab.), and penicillin 50 IU/mL (Sigma Chemical Co., St. Louis, MO) (culture medium). The cells were grown in tissue-culture flasks (75-cm^2 style, Becton Dickinson Labware, Lincoln Park, NJ), which were incubated at 37°C under a humidified atmosphere of 5% CO_2: 95% air, and were passaged once per week into new flasks. For collecting cells from the monolayer in the

flask, we used jet streams of medium (obtained by squeezing medium in and out of a Pasteur pipet) to lift up cells from the surface without any treatment with a proteinase. Afterward, the lifted cells were harvested by centrifugation (700*g* for 5 min). This method eliminates the possibility that receptors may be damaged by enzyme treatment.

One group of cells was treated with PTX (Research Biochemicals International, Natick, MA), a G_i-protein inactivator. PTX was first dissolved in distilled water to make stock solution of 1 mg/10 mL. The stock PTX solution was further diluted in the culture medium to make a 50 ng/mL solution, and GH_4ZR_7 cells were incubated in this PTX-containing medium for 12 h.

Construction of Perifusion Column

A semimicro size disposable cuvet (Dynalon Disposable polystyrene cuvets, 1.5 mL, 10 × 4 × 45 mm, Canlab Scientific Products, Toronto, Canada) was chosen for this study. The lower right corner of the cuvet was drilled, and a piece of silicone tubing (1/23-in. inner diameter, 5/64-in. outer diameter) was inserted into the hole. The lower part of the cuvet was plugged with glass wool (Corning Glass Works, Corning, NY) to prevent leakage of the gel matrix. A matrix is required in the cuvet to attain a perifusion system. Thus, the cuvet was packed with Sephadex gel matrix, which was equilibrated with DMEM containing 0.1% (w/v) BSA (Sigma Chemical Co.) (DMEM-BSA). The cuvet was capped using a rubber stopper, which had been pierced by a piece of 18-gage steel tubing (syringe needle, Becton, Dickinson & Co.), and the pierced stopper was connected to a medium reservoir with a piece of Tygon tubing (R-3603, 1/32-in inner diameter, 3/32-in outer diameter).

Loading of Cells into the Cuvet

Approximately 20 million cells were suspended in 5 mL DMEM in which 10 μL dimethyl sulfoxide (DMSO, Baker Chemical Co., Phillipsburg, NJ) solution containing 1 mmol acetoxymethyl Fluo-3 (Fluo-3, AM; Molecular Probes, Inc., Eugene, OR) and 25% (w/w) Pluronic F-127 were added to make a final concentration of 2 μmol/L Fluo-3, AM. The cells were left incubated for 3 h at room temperature, and then recovered by centrifugation (700*g* for 5 min). The Fluo-3-loaded cells were resuspended in 50 μL DMEM-BSA and were loaded into the prepared cuvet containing 1 mL of Sephadex G-150, which was equilibriated with DMEM-BSA and an addition of 0.2 mL of DMEM-BSA on top of the Sephadex matrix. The cells were injected into the gel matrix between 9 and 18 mm from the bottom of the cuvet, where the excitation light projects in the Perkin-Elmer LS-50 Spectrofluorometer.

Perifusion

Two jacketed columns were used to warm up the control medium (DMEM-BSA) and medium-containing secretagog to 37°C before perifusing through the column. Before the performance of each experiment, DMEM-BSA was pumped out (12000 Vario Perpex, LKB) from the cuvet at a rate of 0.4 mL/min for 20 min to allow cells to adjust to the perifusion environment. During the experimental period, the flow rate was maintained at 0.4 mL/min, whereas either DMEM-BSA or DMEM-BSA containing an appropriate concentration of secretagog was perifused through the cells. At the same time, the perifusate was collected in disposable cups (24 × 14 mm, Sarstedt Canada inc., V-St. Laurent, Quebec) at the rate of 1 sample/minute. The dead spaces between the medium reservoir and the cuvet, and between the cuvet and the fraction collector for the perifusate were 0.3 and 0.9 mL, respectively. At the end of each experiment, the collected fractions were stored at –20°C until assayed.

Fluorometry

The temperature of the cuvet was maintained at 37°C by circulating water through a jacket surrounding the cuvet chamber in the spectrofluorometer. An excitation beam at 490 nm was shone on the cells, and the optical density of the emission light at 540 nm was monitored. Both the excitation and emission slit widths were 10 nm. The relationship between the intensity of fluorescence and $[Ca^{2+}]_i$ was established using the ionomycin and Mn^{2+} quenching technique *(21)* (Fig. 1), and the basal levels of $[Ca^{2+}]_i$ were then computed according to Tsien et al. *(23)* with a $K_d = 320$ n*M* *(39,40)*. The basal $[Ca^{2+}]_i$ in our cells was 114 ± 5 nmol/L. The actual changes of optical density were recorded without converting them to $[Ca^{2+}]_i$.

All perifusion media were prepared immediately prior to use. Frozen TRH (1 mmol/L, Sigma) stock solution was diluted in the control DMEM-BSA medium to make 1 μmol/L solution. DA (DA-HCl, Sigma) was dissolved in appropriate volumes of DMEM-BSA medium to make solutions of different concentrations. Ionomycin (Sigma) was first dissolved in absolute alcohol (5 mmol/L) and then diluted with DMEM to make the appropriate concentrations. A stock solution of 1 mol/L manganese chloride (Mallinckrodt) was made in distilled water and was further diluted in DMEM to make 0.1 mmol/L Mn^{2+} to quench the fluorescence. Finally, 1 mmol/L Mn^{2+} plus 10 μmol/L ionomycin was made with DMEM to quench $[Ca^{2+}]_i$.

Radioimmunoassay

Two hundred microliters of the perifused medium were assayed in duplicates using the radioimmunoassay kit for rat prolactin, which was kindly supplied by A. F. Parlow and P. F. Smith through the Rat Pituitary Hormone Distribution Program. The quantity of prolactin was expressed in terms of NIAMDD rat prolactin RP-3. Coefficients of variation for inter- and intra-assay variability were 14.5 and 7.2%, respectively. The sensitivity was 0.03 ng/tube.

Statistics

Fluo-3 tracings: For each tracing, a straight line was connected between the mean levels before and after secretagog treatment, and considered as the basal level. Changes in $[Ca^{2+}]_i$ were indicated by area surrounded by the tracing between the basal level and the responded level induced by the secretagog treatment. A "cut-and-weigh" method was used to calculate the percentage change in area under the curve (AUC) of the original fluorescent tracing, such that the areas (mg of paper) are expressed in arbitrary units (U^2).

Prolactin data: For each experiment, data for prolactin assays were normalized by considering the average PRL concentration of 15 samples collected before the perifusion of a secretagog-containing medium as the 100% PRL concentration. AUCs in graphs for individual experiments were calculated using the Prism 2.01 program (GraphPad Software Inc., San Diego, CA). Areas were expressed as an arbitrary unit (A^2). AUC of the 7-min immediately prior to secretagog treatment was used as the control. AUC of a 7-min period centering the peak response was taken as the response, since the response of cells to secretagog usually lasted for about 7–10 min.

For both the $[Ca^{2+}]_1$ and prolactin concentration, differences between the experimental groups (control vs treatment) were compared using Student's *t*-test, and a difference was considered to be statistically significant when a *P* value was <0.05.

Acknowledgments

This work was supported by the Medical Research Council of Canada. Authors express their thanks to H. Elsholtz, University of Toronto for providing GH_4ZR_7 cells, and A. F. Parlow and P. F. Smith for supplying the PRL RIA kit through the Nation Hormone and Pituitary Program.

References

1. Neill, J. D. (1988). In *The Physiology of Reproduction*, Knobilland, E. and Neill, J. D. (eds.). Raven, New York, pp. 1379–1390.
2. Ben-Jonathan, N., Arbogast, L. A., and Hyde, J. F. (1989). *Prog. Neurobiol.* **33,** 399–447.
3. Lamberts, S. W. J. and MacLeod, R. M. (1990). *Physiol. Rev.* **70,** 279–318.
4. Shin, S. H. (1978). *Life Sci.* **22,** 67–73.
5. Denef, C., Manet, D., and Dewals, R. (1980). *Nature* **285,** 243–246.
6. Kramer, I. M. and Hopkins, C. R. (1982). *Mol. Cell. Endocrinol.* **28,** 191–198.
7. Burris, T. P., et al. (1992). *Endocrinology* **130,** 926–932.
8. Burris, T. P. and M. E. Freeman (1994). *Proc. Soc. Exp. Biol. Med.* **205,** 226–235.
9. Kineman, R. D., Gettys, T. W., and Frawley, L. S. (1994). *Endocrinology* **135,** 790–793.
10. Chang, A., Shin, S. H., and Pang, S. C. (1998). *Endocrine*, in press.
11. Arey, B. J., et al. (1993). *Proc. Soc. Exp. Biol. Med.* **203,** 60–63.
12. Shin, S. H., et al. (1997). *J. Am. Physiol.*, in press.
13. Stojilkovic, S. S., Izumi, S., and Catt, K. J. (1988). *J. Biol. Chem.* **263,** 13,054–13.061.
14. Lledo, P. M., et al. (1990). *Endocrinology* **127,** 990–1001.
15. William, P., MacVicar, B. A., and Pittman,Q. J. (1990). *J. Neurosci.* **10,** 757–763.
16. Stack, J. and Surprenant, A. (1991). *J. Physiol.* **439,** 37–58.
17. Burris, T. P. and Freeman, M. E. (1993). *Endocrinology* **133,** 63–68.
18. Winiger, B. P., et al. (1987). *Endocrinology* **121,** 2222–2228.
19. Malgaroli, A., et al. (1987). *J. Biol. Chem.* **262,** 13,920–13,927.
20. Wolf, M. E. and Iapatos, G. (1989). *Synapse* **4,** 353–370.
21. Capponi, A. M., et al. (1986). *Methods Enzymol.* **124,** 116–135.
22. Shin, S. H., Soulup, S. C., and Pang, S. C. (1993). *Life Sci.* **53,** 1605–1616.
23. Tsien, R. Y., Pozzan, T., and Rink, T. J. (1982). *J. Cell Biol.* **94,** 325–334.
24. Akerman, S. N., et al. (1991). *Endocrinology* **129,** 475–488.
25. Lew, A. M., Yao, H., and Elsholtz, H. P. (1994). *J. Biol. Chem.* **269,** 12,007–12,013.
26. Lussier, B. T., et al. (1991). *Endocrinology* **128,** 570–582.
27. Schofield, J. G. (1983). *FEBS Lett.* **159,** 79–82.
28. Law, G. J., Pachter, J. A., and Dannies, P. S. (1989). *Mol. Endocrinol.* **3,** 539–546.
29. Luque, E. H., et al. (1986). *Endocrinology* **118,** 2120–2124.
30. Burris, T. P., Stringer, L. C., and Freeman, M. E. (1991). *Neuroendocrinology* **54,** 175–183.
31. Albert, P. R. (1994). *Vitam. Hormones* **48,** 59–109.
32. Dal Toso, R., et al. (1989). *EMBO J.* **8,** 4025–4034.
33. Bunzow, J. R., et al. (1988). *Nature* **336,** .
34. Kineman, R. D., Gettys, T. W., and Frawley, L. S. (1996). *J. Endocrinol.* **148,** 447–455.
35. Lledo, P. M., et al. (1992). *Neuron* **8,** 455–463.
36. Werner, P., et al. (1996). *Mol. Pharmacol.* **49,** 656–661.
37. Login, I. S., Pancrazio, J. J., and Kim, Y. I. (1990). *Brain Res.* **506,** 331–334.
38. Einhorn, L. C., Gregerson, K. A., and Oxford, G. S. (1991). *J. Neurosci.* **11,** 3727–3737.
39. Gregerson, K. A., Chuknyiska, R., and Golesorkhi, N. (1994). *Am. J. Physiol.* **267,** E789–794.
40. Grynkiewiczv, G., Poenie, M., and Tsien, R. Y. (1985). *J. Biol. Chem.* **260,** 3440–3450.