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Dopamine D_2 Receptor Mediates Both Inhibitory and Stimulatory Actions on Prolactin Release

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Dopamine is considered to be the major physiological tonic inhibitor of prolactin release, yet there is increasing evidence showing that it can also stimulate prolactin release from lactotrophs. In primary cultured lactotrophs, the major dopamine receptors responsible for inhibiting prolactin release are dopamine D_2 receptors. A dopamine receptor subtype may be responsible for the stimulatory action, yet one cannot exclude the possibility that a dopamine D_2 receptor can play dual roles. This study was therefore undertaken to investigate if dopamine both stimulates and inhibits prolactin secretion through activation of the same dopamine D_2 receptor. GH_4ZR_7 cells, which have only one type of dopamine receptors—D_{2s}, were perifused with different concentrations of dopamine, and the perifusate was assayed for prolactin; 10^{-7} mol/L dopamine stimulated prolactin release ($p < 0.05$; $n = 5$), whereas 5×10^{-4} mol/L dopamine inhibited prolactin secretion ($p < 0.05$; $n = 5$). In the pertussis toxin-treated cells, 10^{-7} mol/L dopamine stimulated prolactin release ($p < 0.05$; $n = 5$), and 5×10^{-4} mol/L dopamine did not significantly change the rate of prolactin release. These results indicate that both the stimulatory and inhibitory actions of dopamine are likely mediated by the same D_2 receptor subtype, since GH_4ZR_7 cells express only D_{2s} receptors. They also confirm that the inhibitory action of dopamine is mediated through a G_i protein; and the stimulatory action of dopamine is mediated through a PTX-insensitive pathway. These findings suggest that D_2 receptors are coupled to both G_i and G_s proteins.

Key Words: Dopamine; D_2 receptor; prolactin; GH_4ZR_7; pertussis toxin.

Introduction

Prolactin is an important hormone involved in the regulation of a diversity of physiological processes, including milk secretion, reproduction, immune responses, osmoregulation, and growth promotion *(1)*. It is mainly secreted from lactotrophs in the anterior pituitary gland *(2)*, and its release is predominantly under inhibitory control, whereas occasional bursts of prolactin secretion, such as during stress, are likely induced by putative prolactin-releasing factors *(3)*. Dopamine is the major tonic inhibitory regulator of prolactin synthesis and release. It has been considered the physiological prolactin release-inhibiting factor (PIF) *(4)*, yet some studies showed that dopamine can also stimulate prolactin release from lactotrophs *(1,5–9)*. Although the majority of dopamine D_2 receptors in primary cultured lactotrophs are inhibitory *(7,10–12)*, there are one or more D_2 receptor subtypes that act as mediators in the stimulatory action of dopamine *(7,13)*. However, one cannot exclude the possibility that a dopamine D_2 receptor can play dual (stimulatory and inhibitory) roles. We therefore have chosen for this study the GH_4ZR_7 cell line, which, among all dopamine receptor subtypes, has only the transfected D_2 receptors (D_{2s}) *(11)* to find out if activation of dopamine D_2 receptor only inhibits prolactin release or both stimulates and inhibits prolactin secretion.

Results

Thyrotropin releasing hormone (TRH) was used in the study as a reference control to ensure that the cells were responding properly to secretagog. In the GH_4ZR_7 cells, perifusion of TRH (10^{-6} mol/L) caused a significant increase of prolactin release, in both the control and the pertussis toxin-(PTX) treated cells as expected (Fig. 1A,B). The peak prolactin concentration induced by TRH in the nontreated group (51.7 ± 3.3 ng/mL; 268 ± 30% of basal level) (mean ± SEM) (Fig. 1A) was not significantly different from that of the PTX-treated group (49.1 ± 3.4 ng/mL; 263 ± 28% of basal level) (Fig. 1B), indicating that PTX had no effect on the stimulatory action of TRH on prolactin release.

When GH_4ZR_7 cells were treated with 10^{-7} mol/L dopamine, the prolactin release was increased from a basal concentration of 23.3 ± 2.9 ng/mL (100%) (area under the curve [AUC] = 72.7 ± 9.1) to a peak concentration of 31.0 ±1.6 ng/mL or 125 ± 10% of basal concentration (AUC = 102.6 ± 6.9) (mean ± SEM) ($p < 0.05$; $n = 5$) and then returned to a baseline concentration during dopamine

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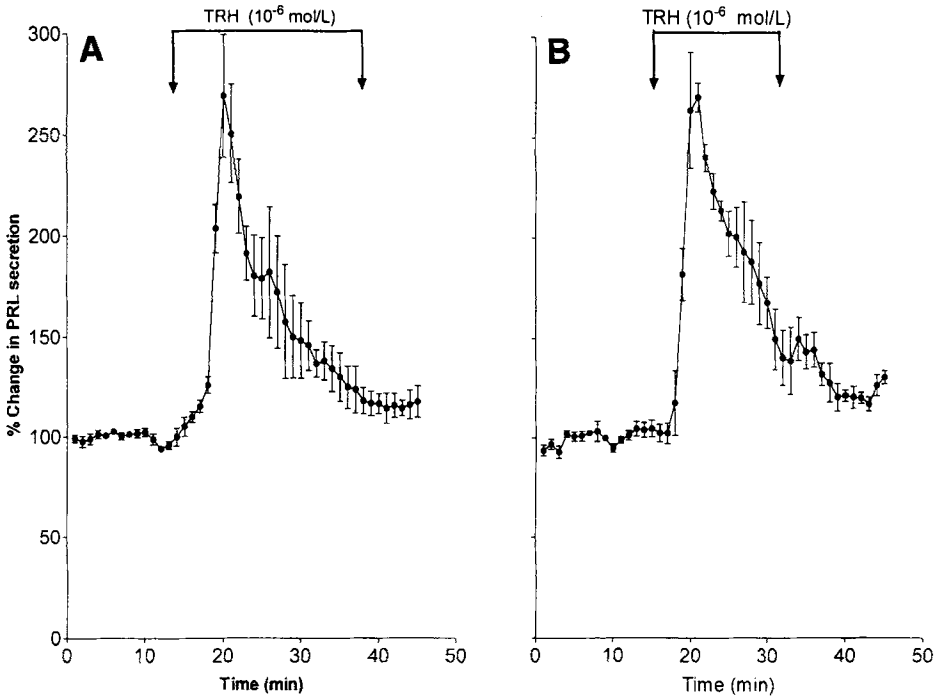

Fig. 1. The effects of TRH on prolactin release from GH_4ZR_7 cells **(A)** and PTX-pretreated GH_4ZR_7 cells **(B)**. Control medium (DMEM-BSA) (fractions # 1–15), TRH (10^{-6} mol/L) (fractions #16–30), and control medium (fractions # 31–45) were perifused through the cells. Each data point represents mean percent change in PRL secretion level (mean ± SEM) of 5 independent experiments (n = 5).

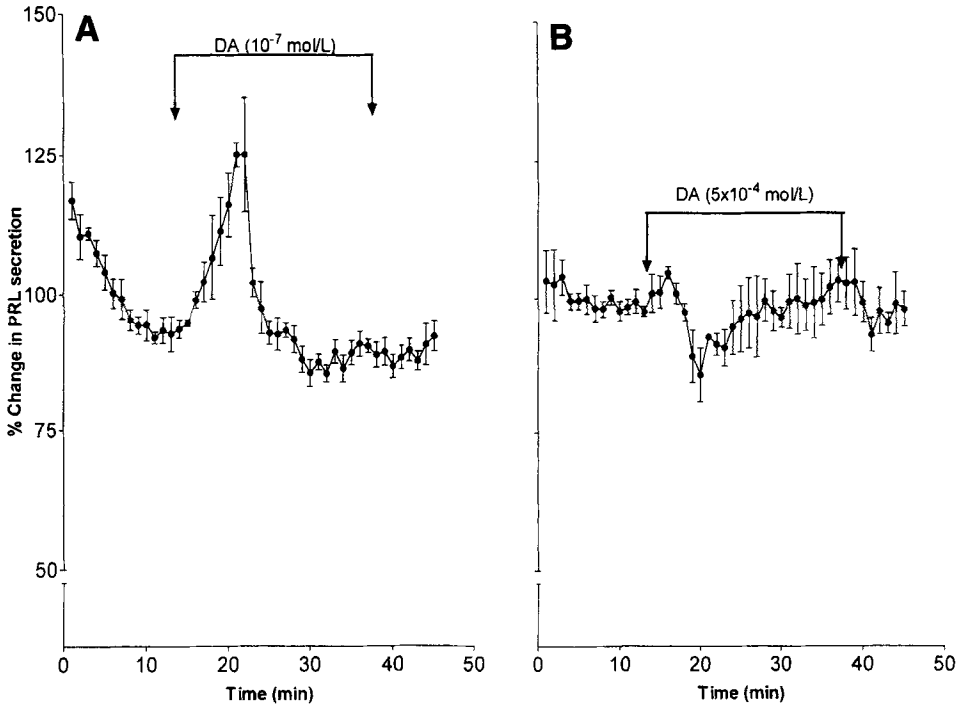

Fig. 2. The effects of 10^{-7} mol/L dopamine (DA) **(A)** and 5×10^{-4} mol/L dopamine (DA) **(B)**, on prolactin release from GH_4ZR_7 cells. Control medium (DMEM-BSA) (fractions #1–15), DA-containing medium (fractions #16–30), and control medium (fractions # 31–45) were perfused through the cells. Each data point represents mean percent change in PRL secretion level (mean ± SEM) of 5 independent experiments (n = 5).

perifusion (Fig. 2A). On the other hand, a higher concentration of dopamine (5×10^{-4} mol/L) decreased prolactin secretion in the GH_4ZR_7 cells. The rate of prolactin secretion was reduced from a basal concentration of 20.0 ± 2.4 ng/mL (100%) (AUC = 60.5 ± 10.1) to nadir concentration of 15.9 ± 1.8 ng/mL or 85 ± 5% of basal level (AUC =

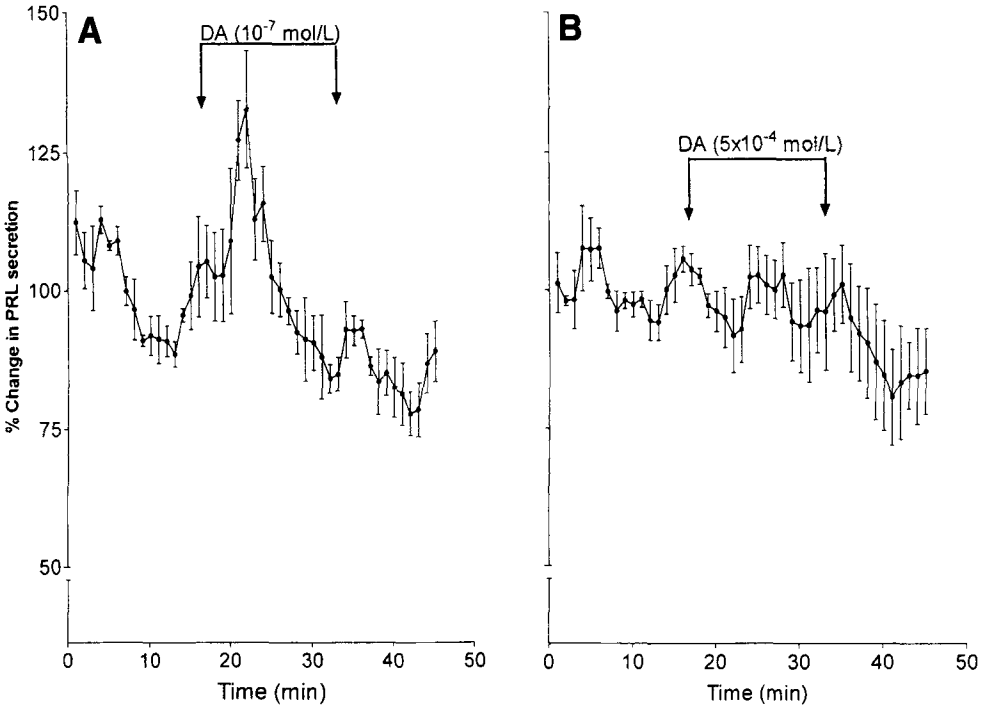

Fig. 3. The effects of 10^{-7} mol/L dopamine (DA) **(A)** and 5×10^{-4} mol/L dopamine (DA) **(B)** on prolactin release from PTX-pretreated GH_4ZR_7 cells. Control medium (DMEM-BSA) (fractions # 1–15), DA-containing medium (fractions #16–30), and control medium (fractions # 31–45) were perfused through the cells. Each data point represents mean percent change in PRL secretion level (mean ± SEM) of five independent experiments ($n = 5$).

44.3 ± 11.2) ($p < 0.05$; $n = 5$) and returned quickly to the basal concentration during dopamine perifusion (Fig. 2B).

In the PTX-treated cells, a low concentration of dopamine (10^{-7} mol/L) significantly stimulated prolactin release. Prolactin concentration was elevated from the basal concentration of 23.5 ± 2.0 ng/mL (100%) (AUC = 74.7 ± 12.3) to a peak concentration of 31.3 ± 2.9 ng/mL—133 ± 10% basal level (AUC = 100.5 ± 13.0) ($p < 0.05$; $n = 5$) (Fig. 3A). In this study, the peak concentration of prolactin (31.3 ± 2.9 ng/mL; basal concentration: 23.5 ± 2.0 ng/mL) induced by dopamine of the PTX-treated group was not significantly different from that of the control (non-PTX-treated) group (peak concentration: 31.0 ± 1.6 ng/mL; basal concentration: 23.3 ± 2.9 ng/mL), indicating that the stimulatory action of DA was not affected by the PTX. A higher concentration of dopamine (5×10^{-4} mol/L) did not significantly changed the rate of prolactin secretion from the PTX-treated cells (Fig. 3B; control: 20.2 ± 1.43 ng/mL [100%]; AUC of control = 64.9 ± 15.5, treatment = 20.2 ± 0.8 ng/mL [97 ± 2%]; AUC of treatment = 66.0 ± 20.2) ($p > 0.5$; $n = 5$). These observations confirmed that the dopamine's inhibitory action is mediated through G_i protein.

Discussion

Our study is the first to establish that the D_{2s} receptor plays a dual role in the control of prolactin secretion in GH_4ZR_7 cells: it mediates a stimulatory signal in the presence of a low dopamine concentration and an inhibitory signal in the presence of a high dopamine concentration.

The synthesis and release of prolactin from lactotrophs have been extensively studied over the past two decades. However, the precise mechanisms that underlie regulation of the secretory pathway are still in large part undefined. Most studies were performed using primary cultured pituitary cells. Generally, they are mixed populations of different lactotroph subtypes *(14)*, and different lactotroph subtypes express different types of dopamine receptors *(15)*. Although these primary cultured cells are more physiologically relevant than cloned cell lines, they cannot illustrate as clearly that a particular dopamine receptor is solely responsible for the stimulatory action.

We used cloned GH_4ZR_7 cells, which originate from the rat pituitary tumor cell line, GH_4C_1, in this study. These cells were selected not only because they are a homogeneous population of lactotrophs, but also because they express only one subtype of dopamine D_2 receptors, namely the D_{2s} receptors *(16)*. GH_4ZR_7 cells are much less sensitive to dopamine's inhibitory action than primary-cultured cells, and the difference between the primary cultured cells and the GH_4ZR_7 cells is likely related to the difference in number of the active membrane-bound receptors on the two cell types. Although dopamine of concentrations between 10^{-7} and 10^{-6} mol/L normally exhibits maximum inhibitory effect on prolactin secretion from primary cultured lactotrophs *(17)*, a much higher dopamine concentration

(10^{-5} mol/L) is required to produce maximum or near-maximum inhibition of prolactin release from GH_4ZR_7 cells *(12)*. In our study, we therefore consider 10^{-7} mol/L dopamine as a low concentration and 5×10^{-4} mol/L as a high concentration.

Prolactin release is mainly regulated by the PIF, believed to be dopamine, which exerts a tonic inhibitory control *(4)*. On the other hand, there are several other hypothalamic peptides that stimulate prolactin release *(4)*, and these prolactin-releasing factors (PRF) could play a physiological role in prolactin release in certain physiological conditions, such as stress *(3)*. Although the chemical identity of the PRF(s) is not yet known, multiple factors are likely involved in stimulating prolactin release. A low concentration of dopamine is one of the possible stimulating factors. Withdrawal of dopamine after its inhibition of prolactin release exhibits a pronounced rebound release of prolactin *(18,19)*. Part of the rebound release may be attributed to the stimulatory action of a decreased concentration of dopamine during the withdrawal process *(15)*. In contrast, there is no rebound release of prolactin when bromocriptine, a powerful dopamine D_2-receptor agonist, is withdrawn from lactotrophs *(18)*. This indicates that rebound release does not occur as a general phenomenon after the inhibitory action of prolactin release is eliminated.

Dopamine at high concentrations inhibits and at low concentrations stimulates prolactin release *(1,5–9)*. A subtype of dopamine receptors (for example, of the D_5 subclass) may be responsible for the stimulatory action *(13)*. Nevertheless, the presence of a stimulatory subtype of dopamine receptors in primary cultured cells does not rule out a possibility that a D_2 receptor can play dual roles. Our work showed that dopamine, depending on its concentration, can either inhibit or stimulate prolactin release in GH_4ZR_7 cells. Since GH_4ZR_7 cells express only a single D_2-receptor subtype, more specifically the short isoform of D_2 receptor (D_{2S}), our results indicate that dopamine both stimulates and inhibits prolactin release through a single dopamine D_2-receptor subtype. Porter et al. *(13)*, however, could not demonstrate stimulatory action of dopamine on prolactin release. The discrepancy between their results and ours may be the result of different experimental conditions. Porter et al. used a static system in their study, but we used a dynamic perifusion system. In a static system, one can only monitor the final accumulated hormone concentration in the medium at the end of the incubation period, and in a dynamic perifusion system, one can monitor moment-to-moment changes of hormone release. Prolactin releases in bursts when a secretagog (e.g., TRH) contacts cells, and a brief burst of hormone secretion can be buried during a static incubation period.

In spite of a continuous stimulation of the GH_4ZR_7 cells with either TRH or dopamine, the response was short-lasting; prolactin concentration returned to basal levels within, on an average, 8 min after either TRH or dopamine was perfused. This kind of transient response is not unique to our cultured cell line, but was also found in the primary cultured pituitary cells *(18)*. This could possibly be owing to the desensitization of dopamine receptors when they were continuously stimulated. Receptors were internalized and downregulated in the presence of the corresponding activators. Thus, the number of functional receptors decreased, and the net response diminished accordingly. When a large number of receptors was desensitized, the cells could no longer respond to the secretagogs, and prolactin secretion returned to normal levels.

The actions of dopamine are very complex and seem to involve multiple G proteins *(7,16,20)* and multiple systems. There is evidence showing that dopamine causes inhibition of adenylyl cyclase activity *(8)*, activation of G protein-regulated potassium channels *(21,22)*, voltage-dependent potassium channels *(23)*, and changes in cytosolic Ca^{2+} ion concentration *(12,24,25)* in lactotrophs. However, the exact mechanisms of dopaminergic actions on prolactin secretion are still not clear. In accordance with previous studies, we confirmed that PTX eliminates the inhibitory action, but does not block the stimulatory action of dopamine (Fig. 2) *(7)*. The inhibitory action is mediated through a G_i protein (possibly $G_{i\alpha 3}$) *(20)*, and the stimulatory action is mediated through a PTX-insensitive pathway (possibly through $G_{s\alpha}$) *(20)*.

The findings of this study suggest that the dopamine D_2 receptors may be coupled to both a G_i and a G_s protein, so that a single receptor is linked to both the inhibitory and the stimulatory pathways. In order to explain the dual role of the D_2 receptors, we propose the following hypothesis: Activation of either of the two pathways depends on the rate of association of dopamine with the receptors; the stimulatory transduction pathway is activated when the rate of association between dopamine and the D_2 receptors is relatively low, and the inhibitory transduction pathway is activated when the rate of association is relatively higher (rate theory). Initial rates of the association are concentration-dependent, assuming that the number of D_2 receptors in an experimental system is constant. In addition, it is possible that both the stimulatory and the inhibitory actions of dopamine are present when lactotrophs are stimulated by dopamine. However, the stimulatory effect can be masked by the inhibitory effect induced by a high concentration of dopamine, and thus only the inhibitory response is observed *(9)*.

In summary, we have demonstrated that activation of a single dopamine D_2 receptor subtype can induce either stimulatory or inhibitory effects on prolactin release, depending on the dopamine concentration in a system, but further studies are required in order to define the exact mechanisms involved in the dopaminergic actions.

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 Dulbecco's Modified Eagles Medium (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, jet streams of medium (obtained by squeezing medium in and out of a Pasteur pipet) were used 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 a 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 semimicrosize 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 G-150, which was equilibrated with DMEM containing 0.1% (w/v) bovine serum albumin (BSA, Sigma Chemical Co.) (DMEM-BSA). The cuvet was capped using a 00-size 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). Approximately 20 million cells were loaded into the prepared column containing 1 mL of Sephadex G-150, which was equilibrated 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 using a Pasteur pipet, and the perifusion column was kept in a water bath at 37°C.

Perifusion

Two jacketed columns were used to warm up the control medium (DMEM-BSA) and secretagog-containing medium to 37°C before perfusing 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 perfused through the cells. At the same time, the perfusate was collected in disposable cups (24 × 14 mm, Sarstedt Canada inc., V-St. Laurent, Quebec) at the rate of 1 sample/min. The dead spaces between the medium reservoir and the cuvet, and between the cuvet and the fraction collector for the perfusate were 0.3 and 0.9 mL, respectively. At the end of each experiment, the collected fractions were stored at –20°C until assayed.

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. Dopamine (dopamine-HCl, Sigma) was dissolved in appropriate volumes of DMEM-BSA medium to make solutions of different concentrations.

Radioimmunoassay

Two hundred microliters of the perfused 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 National Institute of Diabetes and Digestive and Kidney Diseases (NIAMDDK) rat prolactin RP-3. Coefficients of variation for inter- and intraassay variability were 14.5 and 7.2%, respectively. The sensitivity was 0.03 ng/tube.

Statistics

Areas under the curve (AUC) in the graphs for the individual experiments were calculated using the Prism 2.01 program (GraphPad Software Inc., San Diego, CA). Areas were expressed as an arbitrary unit (U^2). The control for each treatment in each individual experiment was taken as AUC of the 7-min period immediately prior to secretagog treatment. For each treatment, AUC of a 7-min period—3 min prior to and 3 min after the peak response—was taken since the response of cells to secretagog usually lasted for about 7–10 min. Differences between the experimental groups (control vs treatment) were compared using paired Student's *t*-test, and a difference was considered to be statistically significant when a *P*-value was <0.05.

Acknowledgments

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