Stimulation of the release of [32 P]guanosine 5'-diphosphate from G proteins in the rat anterior pituitary lobe by gonadotrophin-releasing and thyrotrophin-releasing hormones

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Summary. Plasma membranes (1–2 mg protein) purified from the anterior pituitary lobes of adult male rats were incubated with 0.6 μmol [α - 32 P]guanosine 5'-triphosphate (GTP) l^{-1} in an ATP-regenerating buffer at 37°C for 60 min; during this incubation the [32 P]GTP was hydrolysed and the nucleotide that was predominantly bound to the membranes was [32 P]GDP. The release of [32 P]GDP from the membranes was monitored at 37°C; the amount released was proportional to the protein concentration and increased as a function of time. 5'-Guanylylimidodiphosphate (Gpp(NH)p) increased [32 P]GDP release by up to 30% at 0.1 μmol l^{-1} . Although 10 nmol Gpp(NH)p l^{-1} had no effect on GDP release, it appeared to stabilize the hormonal effect by blocking further GDP–GTP exchange.

Gonadotrophin-releasing hormone (GnRH) agonist and thyrotrophin-releasing hormone (TRH), at 0.1 μmol l^{-1} caused a maximum increase in the release of [32 P]GDP of 31–38%. The GnRH agonist (0.1 μmol l^{-1}) stimulated GDP release by 21%, 24%, 17% and 14% at 30 s, 1, 2 and 5 min, respectively. TRH (0.1 μmol l^{-1}) stimulated GDP release by 38%, 30%, 17% and 16% at 30 s, 1, 2 and 5 min, respectively. A GnRH antagonist also stimulated [32 P]GDP release, albeit less effectively than GnRH agonist; the antagonist did not inhibit agonist stimulation of GDP release. These results indicate that ligand binding to the GnRH and TRH receptors results in interaction of the receptor with a guanine-nucleotide-dependent transducer protein (G protein) and activation of GTP–GDP exchange. A receptor antagonist (at the GnRH receptor) has partial efficacy at eliciting this response. This GDP release represents an early biochemical marker of GnRH and TRH receptor stimulation.

Keywords: G proteins; pituitary; gonadotrophin-releasing hormone; thyrotrophin-releasing hormone; rat

Introduction

Gonadotrophin-releasing hormone (GnRH) is synthesized in the hypothalamus and acts on the anterior lobe of the pituitary to stimulate secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These gonadotrophins control ovarian and testicular steroidogenesis and gamete maturation (Huckle & Conn, 1988; Clayton, 1989; Naor, 1990). Thyrotrophin-releasing hormone (TRH) is also synthesized in the hypothalamus and plays an important role in regulating the pituitary–thyroid axis (Gershengorn, 1986; Hinkle, 1989). TRH stimulates the secretion and synthesis of thyroid stimulating hormone (TSH) and prolactin from the anterior pituitary lobe.

Hormone secretion in response to GnRH or TRH involves phospholipid hydrolysis and calcium mobilization. Consistent effects of TRH and GnRH on adenylate cyclase activity have not been demonstrated (Gershengorn, 1986; Huckle & Conn, 1988; Clayton, 1989; Hinkle, 1989; Noar, 1990).

Andrews *et al.* (1986) observed that guanyl nucleotides stimulated LH secretion and inositol phosphate accumulation in rat anterior pituitary cell cultures, and that these effects were blocked by a GnRH antagonist. Limor *et al.* (1989) reported that guanosine 5'-triphosphate (GTP) and guanosine 5'-thiophosphate (GTP γ S) decreased the affinity of pituitary membranes for GnRH and stimulated LH secretion in rat anterior pituitary cell cultures. Perrin *et al.* (1989) demonstrated that guanyl nucleotides inhibited the binding of an 125 I-labelled GnRH agonist to bovine anterior pituitary membranes without affecting the binding of an 125 I-labelled GnRH antagonist. GTP and its analogues also inhibit [3 H]TRH binding to pituitary cell membranes by decreasing receptor affinity (Taylor & Burt, 1981; Hinkle & Kinsella, 1984). The effects of TRH on phospholipid hydrolysis and concentration of cytosolic free calcium are potentiated by GTP and its analogues (Lucas *et al.*, 1985; Martin *et al.*, 1986; Straub & Gershengorn, 1986). In GH $_3$ and GH $_4$ C $_1$ cells, TRH stimulated the low K_m GTPase activity (EC 3.6.1.-) associated with G proteins (Hinkle & Phillips, 1984; Wojcikiewicz *et al.*, 1986; Winicov *et al.*, 1990). We have recently standardized an assay to monitor GTP γ S binding to rat anterior pituitary membranes, and used this method to demonstrate that GnRH and TRH stimulate GTP γ S binding (Ravindra & Aronstam, 1990a); these two hormones also stimulate G protein GTPase activity (Ravindra & Aronstam, 1990a). Taken together, these results (Taylor & Burt, 1981; Hinkle & Kinsella, 1984; Hinkle & Phillips, 1984; Lucas *et al.*, 1985; Andrews *et al.*, 1986; Gershengorn, 1986; Martin *et al.*, 1986; Wojcikiewicz *et al.*, 1986; Limor *et al.*, 1989; Perrin *et al.*, 1989; Ravindra & Aronstam, 1990a; Winicov *et al.*, 1990) indicate that a guanine-nucleotide-dependent regulatory protein (G protein) is functionally coupled to the GnRH and TRH receptor recognition site in pituitary membranes.

G proteins are a family of closely related proteins involved in the transfer of information from surface receptors to biochemical effector mechanisms in a variety of systems (Gilman, 1987; Spiegel *et al.*, 1988; Birnbaumer *et al.*, 1990; Iyengar & Birnbaumer, 1990). G proteins are heterotrimers, containing a variable α subunit (39–46 kDa) in which resides the specificity for receptor and effector mechanisms, and β and γ subunits (35–36 and 8 kDa, respectively) which may play regulatory roles. Several types of G proteins have been identified: G $_s$ mediates stimulation of adenylate cyclase by β -adrenergic (and other) receptors; G $_i$ mediates inhibition of adenylate cyclase by muscarinic (and other) receptors.

To assess the coupling of hormone-receptor with G protein further, we examined the effect of GnRH and TRH on [32 P]GDP release in plasma membranes purified from the anterior pituitary lobes from adult male rats. GDP release is considered to be the rate limiting step in the G protein transduction cycle, and it is this step that is stimulated by the interaction of G proteins with the activated receptor (Gilman, 1987).

Materials and Methods

Materials

GnRH agonist (p Glu-His-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-Gly-NH $_2$); (catalogue no. L-0512), GnRH antagonist (p Glu-D-Phe-Pro-Ser-Tyr-D-Phe-Leu-Arg-Pro-Gly-NH $_2$); (catalogue no. L-4261), TRH (catalogue no. P-2161), and polyethyleneimine-cellulose, thin layer chromatography (TLC) plates (catalogue no. T-6765) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Radiolabelled guanosine-5'-triphosphate ($[\alpha\text{-}^{32}\text{P}]$; 1500 Ci mmol $^{-1}$) was purchased from ICN Radiochemicals (Irvine, CA, USA). All other chemicals were purchased from Sigma Chemical Co.

Anterior pituitary membrane preparation

Plasma membranes were prepared according to the method of Hubbard *et al.* (1983), with some modifications. Adult, male Wistar rats (125–150 g, Harlan Sprague-Dawley, Indianapolis, IN, USA) were housed in pairs in an

environmentally controlled room with free access to food and water. Rats were killed by decapitation and their anterior pituitary lobes removed. All the subsequent procedures were conducted at 4°C. Routinely, tissue from 30–40 rats was homogenized in buffer A (5 mmol Tris-HCl l⁻¹, 1 mol EDTA l⁻¹, 1 mol dithiothreitol l⁻¹, pH 7.4) using a motor-driven Teflon–glass tissue grinder. The homogenate was centrifuged at 800 g for 20 min. The supernatant was maintained on ice while the pellet was resuspended in buffer A and centrifuged at 800 g for 20 min. The combined supernatants were then centrifuged at 100 000 g for 60 min. The pellet was resuspended in buffer A containing 0.7 mol sucrose l⁻¹ and overlaid on to a discontinuous sucrose gradient and spun at 60 000 g for 60 min; the gradient consisted of 4.5 ml of 1.3 mol l⁻¹ sucrose buffer A, 2.5 ml of 1.0 mol sucrose-buffer A l⁻¹, and 2.5 ml of sample. After centrifugation, 2.5 ml from the top of each tube was carefully collected, pooled, washed with buffer A and stored frozen at –80°C. Protein content was estimated using the Bradford method (1976).

Labelling of plasma membranes with [³²P]GTP

Plasma membranes (1–2 mg protein) were incubated in an ATP-regenerating buffer (pH 7.4) containing 75 mmol Tris-HCl l⁻¹, 2 mmol MgCl₂ l⁻¹, 0.5 mmol ATP l⁻¹, 0.5 mmol App(NH)p l⁻¹, 5 mmol phosphocreatine l⁻¹, creatine phosphokinase (50 units ml⁻¹), 50 µg bovine serum albumin, 0.1 mmol EDTA l⁻¹, 0.2 mmol EGTA l⁻¹, 1 mmol cAMP l⁻¹, 100 mmol NaCl l⁻¹, and 0.6 µmol [α-³²P]GTP l⁻¹, in a volume of 100 µl at 37°C for 60 min (Cassel & Selinger, 1978). The membranes were diluted with 10 ml ice-cold TED buffer (10 mmol Tris-HCl l⁻¹, 1 mmol EDTA l⁻¹, 100 mmol NaCl l⁻¹ and 1 mmol DTT l⁻¹, pH 7.4) and washed five times with the same buffer by centrifugation (48 000 g for 45 min at 4°C). The pellet was resuspended in 400 µl of TED buffer and immediately used in the GDP release assay.

[³²P]GDP release assay

The ³²P-labelled plasma membranes (5–20 µg protein) were incubated in triplicate in TED buffer in a volume of 100 µl at 37°C for 5 min. The reaction was terminated by transferring the tubes to an ice bath, followed by centrifugation at 48 000 g for 45 min. An aliquot (10 µl) from the sample was spotted on TLC plates and developed with 1.5 mol LiCl l⁻¹ (Cassel & Selinger, 1978). Spots corresponding to GTP, GDP and GMP (identified using standards) were cut, placed in a scintillation vial containing 6 ml Scintiverse BD (Fisher Scientific, Pittsburgh, PA, USA) and the radioactivity content determined using a Beckman (Palo Alto, CA, USA) liquid scintillation counter.

Statistical analysis

Statistical differences among various groups were determined by analysis of variance and Fisher and Scheffe tests using the StatView II program.

Results

Preliminary studies

As the conditions for the GDP release assay in the anterior pituitary lobe have not been reported in the literature, we conducted preliminary studies to standardize and validate this assay for use with rat anterior pituitary lobe. Incubation of pituitary membranes with [α-³²P]GTP in an ATP-regenerating buffer results in the hydrolysis of GTP by G proteins, and, therefore, the nucleotide that is predominantly bound to the membranes after the labelling step is GDP. Membranes (10–20 µg protein) were incubated with 1 µmol Gpp(NH)p l⁻¹, a non-hydrolysable analog of GTP, and [³²P]GDP release was monitored. Chromatographic analysis revealed that 77% of the [³²P]-labelled nucleotides released was GDP; GTP and GMP accounted for 17% and 6%, respectively (data not shown). The amount of [³²P]GDP released was proportional to the protein content of the assay medium (Fig. 1a).

Influence of Gpp(NH)p on stimulation of release of [³²P]GDP by TRH and GnRH

Gpp(NH)p (0.1 µmol l⁻¹) maximally stimulated [³²P]GDP release by about 30% (*P* < 0.05; Fig. 1b). When the effect of GnRH (1 µmol l⁻¹) and TRH (1 µmol l⁻¹) on [³²P]GDP release was examined in the presence of Gpp(NH)p (1 µmol l⁻¹), [³²P]GDP release was maximally stimulated by only 30%, indicating that potentiation in the presence of both TRH (or GnRH) and Gpp(NH)p

Fig. 1. Release of [^{32}P]guanosine 5'-diphosphate (GDP) from labelled rat pituitary membranes incubated in Tris-HCl buffer (pH 7.4) for 5 min at 37°C was determined by thin layer chromatography. Each point represents the mean $\pm$ SD of three determinations from one experiment. *Values significantly different from control level ($P < 0.05$). This experiment was conducted twice with similar results. (a) Effect of protein concentration on [^{32}P]GDP was determined in the presence of $1 \mu\text{mol}$ 5'-guanylimidodiphosphate (Gpp(NH)p) l^{-1} . (b) Effect of Gpp(NH)p on [^{32}P]GDP release from labelled pituitary membranes ($10 \mu\text{g}$ protein).

did not occur (data not shown). As these results did not allow us to evaluate the extent of the hormone-stimulated GDP release, experiments were conducted with either GnRH or TRH alone in the absence of Gpp(NH)p. Although both of these hormones stimulated [^{32}P]GDP release, this effect was quite variable (data not presented). When 10 nmol Gpp(NH)p l^{-1} was included in the reaction mixture, reproducible and concentration-dependent stimulation with either TRH or GnRH was observed. TRH, 10 nmol l^{-1} , $0.1 \mu\text{mol l}^{-1}$ and $1 \mu\text{mol l}^{-1}$, stimulated [^{32}P]GDP release by 20%, 32% and 36%, respectively ($P < 0.05$ at all points; Fig. 2a); GnRH stimulation of GDP release was 18%, 22%, 29% and 31% at 1 nmol l^{-1} , 10 nmol l^{-1} , $0.1 \mu\text{mol l}^{-1}$, and $1 \mu\text{mol l}^{-1}$, respectively ($P < 0.05$ at all points; Fig. 2b). Although at 10 nmol l^{-1} Gpp(NH)p had no effect

Fig. 2. Stimulation of release of [^{32}P]GDP by (a) thyrotrophin-releasing hormone (TRH) and (b) gonadotrophin-releasing hormone (GnRH). Labelled pituitary membranes ($10 \mu\text{g}$ protein) were incubated with various concentrations of GnRH or TRH and in the presence of 10 nmol 5'-guanylimido-diphosphate (Gpp(NH)p) l^{-1} for 5 min at 37°C . Each point represents the mean $\pm$ SD of three determinations from one experiment. *Values significantly different from control level ($P < 0.05$); this experiment was conducted four times with similar results.

on GDP release (Fig. 1b), it appeared to stabilize the hormonal effect. Hormone binding to its receptor results in receptor–G-protein interaction and the subsequent release of GDP from the G protein. Since the affinity of Gpp(NH)p for G proteins is greater than that of GDP, the vacant nucleotide site (as a result of hormone-induced GDP release) is rapidly occupied by Gpp(NH)p and further GDP–GTP exchange is blocked.

Time course of hormone effect on release of [32 P]GDP

A time course study revealed that $0.1 \mu\text{mol l}^{-1}$ TRH stimulated [32 P]GDP release by 38%, 30%, 17% and 16% at 30 s, 1, 2 and 5 min, respectively ($P < 0.05$ at all points; Fig. 3a); similarly, GnRH ($0.1 \mu\text{mol l}^{-1}$) stimulated [32 P]GDP release by 21%, 24%, 17% and 14% at 30 s, 1, 2 and 5 min, respectively ($P < 0.05$ at all points; Fig. 3b).

Fig. 3. Time course of [32 P]GDP release in the presence of (a) thyrotrophin-releasing hormone (control, ○; TRH, ●) and (b) gonadotrophin-releasing hormone (control, ○; GnRH, ●). Labelled pituitary membranes ($10 \mu\text{g}$ protein) were incubated with $0.1 \mu\text{mol l}^{-1}$ GnRH or TRH in the presence of $10 \text{ nmol Gpp(NH)p l}^{-1}$. Each point represents the mean $\pm$ SD of three determinations from one experiment. *Values significantly different in the presence and absence of hormone at this time ($P < 0.05$); this experiment was conducted twice with similar results.

Effect of GnRH antagonist on [32 P]GDP release

A GnRH antagonist maximally stimulated [32 P]GDP release by up to about 20% at 10 nmol l^{-1} ($P < 0.05$; Fig. 4a). The effect of various concentrations of antagonist was investigated in the presence or absence of 100 nmol l^{-1} agonist, and it was observed that the antagonist did not block the agonist effect (Fig. 4a). In another experiment, the effect of various concentrations of agonist was examined in the presence or absence of 100 nmol l^{-1} antagonist; agonist stimulation was unaffected by the antagonist (Fig. 4b).

Discussion

Hormone secretion in response to either GnRH or TRH involves a series of biochemical events, including: (i) GnRH or TRH binding to its receptor, (ii) receptor–G-protein interaction, (iii) stimulation of second messenger formation and (iv) exocytosis of LH, FSH or PRL secretory granules. The involvement of G proteins in signal transduction at GnRH and TRH receptors follows from the demonstration that TRH stimulates the low K_m GTPase activity in GH_4C_1 and GH_3 cell

Fig. 4. Labelled pituitary membranes ($10 \mu\text{g}$ protein) were incubated with various concentrations of gonadotrophin-releasing hormone (GnRH) antagonist or agonist in the presence of $10 \text{ nmol } 5'$ -guanylylimido-diphosphate (Gpp(NH)p) l^{-1} for 5 min at 37°C . Each point represents the mean $\pm$ SD of three determinations from one experiment. This experiment was conducted twice with similar results. *Values significantly different from control level ($P < 0.05$). (a) Effect of various concentrations of GnRH antagonist with and without the agonist ($100 \text{ nmol } \text{l}^{-1}$). The amount of [^{32}P]GDP released in the presence of 100 nmol agonist l^{-1} (with no antagonist) is $451 \pm 18 \text{ c.p.m. } \mu\text{g}^{-1} \text{ protein}$. Antagonist, ($\bigcirc$); antagonist + agonist, ($\bullet$). (b) Effect of various concentrations of GnRH agonist with and without the antagonist ($100 \text{ nmol } \text{l}^{-1}$). The amount of [^{32}P]GDP release in the presence of 100 nmol antagonist l^{-1} (with no agonist) is $78 \pm 6 \text{ c.p.m. } \mu\text{g}^{-1} \text{ protein}$. Agonist, ($\bigcirc$); agonist + antagonist, ($\bullet$).

membranes (Hinkle & Phillips, 1984; Wojcikiewicz *et al.*, 1986; Winicov *et al.*, 1990), and that TRH and GnRH stimulate GTP γ S binding and G protein GTPase activity in the rat anterior pituitary membranes (Ravindra & Aronstam, 1990a). According to the generally accepted model of G protein function (Gilman, 1987), the binding of TRH or GnRH to its receptor facilitates an exchange of GTP for GDP on the α subunit. The rate limiting step of the cycle is the release of GDP from the α subunit, and it is this step that is stimulated by G-protein-receptor interaction (Gilman, 1987). The activated α_{GTP} subunit dissociates from the $\beta\gamma$ subunits, and interacts with effector molecules such as phospholipase C. An intrinsic GTPase activity of the α subunit hydrolyses GTP to GDP, releasing inorganic phosphate (Pi); α_{GDP} then recombines with $\beta\gamma$, ending the activation cycle. Hormone stimulation of GDP release has not been demonstrated in the pituitary, or in any other endocrine system.

In this study we have demonstrated that binding of GnRH or TRH to plasma membranes stimulates [^{32}P]GDP release. A statistically significant stimulation was observed at 1 nmol GnRH l^{-1} and 10 nmol TRH l^{-1} . Using purified plasma membranes, we observed stimulation of GTP γ S binding and G protein GTPase activity at 1 – $10 \text{ nmol } \text{l}^{-1}$ GnRH or TRH (data not shown). In both of these receptor systems, stimulation of GDP release, GTP γ S binding and GTPase activity was about 15–40% (present study), 50–65%, and 60–90%, respectively (data not shown). Using a crude membrane preparation, we observed stimulation of GTP γ S binding and GTPase activity at 0.1 – $1 \mu\text{mol } \text{l}^{-1}$ GnRH or TRH; GTP γ S binding and GTPase activity were maximally stimulated by about 25–35% and 40–50%, respectively (Ravindra & Aronstam, 1990a). Stimulation of [^{32}P]GDP release, however, was not consistently observed with the crude membrane preparation. The use of purified plasma membranes is clearly better for examining specific steps in the G protein cycle in the rat anterior pituitary lobe.

Since the GDP release step might represent the first biochemical event subsequent to hormone binding, the modest amount of stimulation of [^{32}P]GDP release by GnRH or TRH is consistent with our present knowledge of the signal transduction process; a small amount of G protein stimulation can lead to a full hormonal response. For example, Andrews *et al.* (1986) observed that

0.1 $\mu\text{mol GnRH l}^{-1}$ stimulated inositol phosphate accumulation in rat pituitary cells by about 800% and LH secretion by approximately 2000%. In GH_3 cells, 1 $\mu\text{mol TRH l}^{-1}$ maximally stimulated the inositol phosphate accumulation by about 900% (Wojcikiewicz *et al.*, 1986). Thus, it appears that the signal is amplified at successive steps.

The variability of GnRH and TRH stimulation of [^{32}P]GDP release between experiments (15–40%) might be a reflection of the dynamic process of GDP–GTP exchange cycle. Hormone binding to its receptor results in receptor–G-protein interaction and the subsequent release of GDP from the G protein. However, Gpp(NH)p (a nonhydrolysable GTP analogue) can also release the bound [^{32}P]GDP and block further GDP–GTP exchange. Addition of 10 nmol Gpp(NH)p l^{-1} to the reaction mixture, a concentration at which this compound did not stimulate [^{32}P]GDP release, appeared to decrease the variability of the hormonal stimulation.

In turkey erythrocytes (Cassel & Selinger, 1978) and rat adipocytes (Murayama & Ui, 1984), isoproterenol-stimulated [^3H]GDP release was potentiated by Gpp(NH)p. Similarly, in rod outer segment membranes, light-induced [^3H]GDP release was potentiated by GTP (Fung & Stryer, 1980). In contrast, potentiation of hormone-stimulated [^{32}P]GDP release by Gpp(NH)p was not observed in pituitary membranes. The effect of submaximal concentrations of Gpp(NH)p and either TRH or GnRH was also examined, and we did not observe any potentiation (data not shown). The reason for this difference is not clear.

In previous studies, micromolar concentrations of agonist were present during labelling of membranes with [^3H]GTP, resulting in an increased incorporation of radioactivity by the membranes. However, the membranes were desensitized to subsequent exposure to the agonist, as indicated by the fact that stimulation of GDP release required 50–100 $\mu\text{mol agonist l}^{-1}$ (Cassel & Selinger, 1978; Murayama & Ui, 1984). In preliminary experiments, when either GnRH or TRH was included in the labelling step, micromolar concentrations of the hormones were required to stimulate [^{32}P]GDP release (data not shown). We therefore, labelled the pituitary membranes with [α - ^{32}P]GTP in the absence of GnRH or TRH. The membranes were not refractory to the hormones during the GDP release step. A statistically significant stimulation of GDP release was observed at 1 nmol GnRH l^{-1} or 10 nmol TRH l^{-1} .

In the absence of hormone, considerable 'passive' GDP–GTP exchange appears to take place in the anterior pituitary lobe. It is unclear how this 'passive' exchange is regulated. One group of molecules that could regulate this process are ions such as Mg^{2+} or Ca^{2+} . Other possible modulators are proteins (other than transducer G proteins) present in the plasma membranes that are also capable of GTP–GDP exchange. One such protein is tubulin and its presence has been demonstrated in association with rat brain membranes (Bhattacharyya & Wolff, 1975). Binding of tubulin to a G protein affinity column (Higashi & Ishibashi, 1985) and to G_{sa} and G_{ia} has been reported (Wang *et al.*, 1990). Anti-tubulin antibodies, especially anti- β -tubulin antibody, stimulated the low K_m GTPase activity associated with G proteins in rat striatal membranes and potentiated stimulation of this activity by acetylcholine. This suggests that tubulin acts as a GTP sink and exerts a chronic inhibitory influence on G protein function or receptor–G-protein interactions (Ravindra & Aronstam, 1990b). Future work should reveal if similar regulatory mechanisms are present in the anterior pituitary lobe.

GnRH antagonist stimulated GDP release by about 20%. It appears that the binding of any ligand to the GnRH recognition site is sufficient for the initial promotion of receptor–G-protein interaction and activation of the G protein cycle. The distinction between agonist and antagonist may lie in the persistence of the activation (a function that was not measured in the present work). Analogous observations have been reported in other receptor systems. For example, at nicotinic cholinergic receptors, both agonists and antagonists increase the exposure of sites within the ionophore to noncompetitive blockers, an action that is thought to reflect opening of the ionophore (Aronstam *et al.*, 1981). It is also possible that the stimulation by antagonists is only transient, and that the receptor is subsequently left in a blocked state (antagonists generally dissociate more slowly from receptors than agonists – a property that may underlie their inhibitory potential). It is

important to note that only an initial effect on [32 P]GDP release from the prelabelled G protein would be detectable using the present assay. Appreciation of antagonist actions might require monitoring G protein cycle turnover over an extended period of interaction.

We have previously demonstrated that the same GnRH antagonist stimulated the rat pituitary G protein GTPase activity. Although agonist-stimulated activity was decreased at higher antagonist concentrations, a complete inhibition of the agonist effect was not observed (Ravindra & Aronstam, 1990a). In the present study, the antagonist did not inhibit the agonist-stimulated [32 P]GDP release. This antagonist was previously observed to block, almost completely, GnRH-stimulated LH secretion by rat anterior pituitaries *in vitro* (Humphries *et al.*, 1978). It was previously observed that a GnRH antagonist blocked agonist-stimulated inositol phospholipid turnover (Andrews *et al.*, 1986). Taken together, these results suggest that the antagonist effect on GDP release and GTPase activity are not coupled to either inositol phosphate accumulation or LH secretion. It is possible that GnRH antagonist binding to the receptor might result in its coupling to an inhibitory G protein. If, for example, the binding of a GnRH antagonist to the receptor results in its coupling to an inhibitory G protein, it could still lead to a stimulation in GTPase activity; this enzyme activity is only an indication of an end to this particular G protein cycle. Further delineation of these initial steps in the signal transduction pathway can be achieved only by purification of these proteins from the anterior pituitary lobe.

We demonstrated that GnRH and TRH stimulate [32 P]GDP release in plasma membrane fraction from the rat anterior pituitary lobe. These results indicate that a G protein is functionally coupled to the GnRH or TRH receptor recognition site in pituitary membranes. [32 P]GDP release represents an early biochemical marker of ligand occupancy of GnRH and TRH receptors.

This work was supported by PHS grants GM-37948 and AA-07698 and Biomedical Research Support Grants program of Medical College of Georgia. We would like to express our sincere gratitude to C. Marquis for unstinted and excellent help in the preparation of this manuscript.

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Received 8 May 1991