Neurotransmitter Receptor Agonists Regulate Growth Hormone Gene Expression in Cultured Ovine Pituitary Cells (43786)

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Abstract. **The regulation of growth hormone (GH) secretion and GH mRNA content by the dopaminergic agonist, bromocriptine (BRO); the β-adrenergic agonist; isoproterenol (ISO); the α_1-adrenergic agonist, methoxamine (MET); the α_2-adrenergic agonist, clonidine (CLON); the serotonergic agonist, quipazine (QUIP); somatostatin (SS) and GH-releasing hormone (GHRH) were studied using cultured ovine anterior pituitary cells. Clonidine and BRO (10^{-6} *M*) inhibited basal and GHRH (10^{-10} *M*)-stimulated GH release. Bromocriptine enhanced GH mRNA content and potentiated the GHRH (10^{-8} *M*)-stimulated content of GH mRNA, while CLON had no effect on GH mRNA. Quipazine had little effect on GH secretion and no effect on GH mRNA content. Methoxamine and ISO (10^{-6} *M*) increased basal secretion of GH and both enhanced GHRH-stimulated GH secretion. Both MET and ISO increased GH mRNA content of cultured ovine pituitary cells. Somatostatin (10^{-7} *M*) inhibited GHRH-stimulated GH secretion and GH mRNA accumulation. These results support the hypothesis that neurotransmitters may regulate or interact to further modulate pituitary hormone release. Moreover, the data indicate that neurotransmitters may not only regulate secretion but also regulate GH mRNA content and thus affect hormone synthesis.**

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Growth hormone (GH) secretion from the somatotrope is directly regulated by the stimulatory actions of growth hormone-releasing hormone (GHRH) and the inhibitory effects of somatostatin (SS). However, other factors may modulate GH secretion either by acting on the release of these neuropeptides at the hypothalamic level or by interacting with these peptides at the level of the anterior pituitary (1, 2).

Neurotransmitters stimulate GH release by acting at sites in the hypothalamus and altering release of GHRH or SS or regulate GH release directly from the pituitary gland (3). Recent studies provide evidence for neurotransmitters reaching the anterior pituitary gland. Epinephrine is found in the portal veins of rats (4); the source likely is the adrenal gland. Norepinephrine is also found in the portal veins in concentrations greater than peripheral plasma of the sheep (5). Dopamine was not found in the portal veins of sheep, but dopamine from the posterior pituitary gland was found to be in sufficient concentrations to provide tonic regulation of prolactin release (6). Other studies have directly investigated the role of neurotransmitters on anterior pituitary function using cultured pituitary cells. These findings indicate the presence of neurotransmitter receptors on cells of the anterior pituitary and a role for neurotransmitters in regulating GH release (7–14). Although numerous studies have demonstrated regulation of GH release, there is little evidence for the

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possibility of neurotransmitter receptors regulating GH gene expression. Therefore, this study sought to determine whether GH mRNA content could be altered by the presence of neurotransmitter receptor agonists and whether these agonists could modify the actions of GHRH on GH mRNA content.

Materials and Methods

Animals. Crossbred (Suffolk × Dorset), castrate, male sheep from 1 to 1½ years of age and weighing between 30 and 50 kg were used for these studies. All sheep were maintained on pasture and supplemented with coastal bermudagrass hay, grain, and minerals daily, and provided water *ad libitum*.

Cell Culture. Each sheep was euthanized with sodium pentobarbital and the whole pituitary removed and placed into sterile HEPES buffered saline solution (137 m*M* NaCl, 5 m*M* KCl, 0.7 m*M* Na_2HPO_4, 25 m*M* HEPES, 10 m*M* glucose, 0.36 m*M* $CaCl_2$, 3% bovine serum albumin fraction V [BSA]; Sigma Chemical Co., St. Louis, MO) supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.03 μg/ml fungizone (Gibco BRL, Inc., Grand Island, NY). The pituitary was placed in an ice bath and immediately transported to the laboratory. The anterior pituitary was dissected from the whole pituitary at room temperature and placed in HEPES-saline under a laminar flow hood. The tissue was minced, rinsed three times in HEPES-saline and dispersed (15, 16) using 6 ml of a solution containing 0.35% collagenase (Type 1a), 0.1% hyaluronidase in HEPES-saline, and incubated for 45 min at 30°C. After enzymatic digestion, the tissue was triturated 35–40 times by passage through a 10-ml pipette. Cells were filtered through sterile presoaked gauze and pelleted by centrifugation at 800 rpm for 10 min at 30°C. The cell pellet was washed six times in Dulbeccos Modified Eagles Medium (D-MEM; Gibco BRL, Inc.) supplemented with 10% heat-inactivated, charcoal-stripped fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 0.30 μg fungizone, and 2 m*M* glutamine. Typically, one sheep anterior pituitary yielded 18×10^6 cells with 89%–99% viability as determined by trypan blue exclusion. The cells were plated at a density of 10^6 cells/35 mm well (Corning, Corning, NY) on Day 0 and incubated at 37°C in a humidified atmosphere of 95% air, 5% CO_2. Three days later, cells were washed three times in serum-free D-MEM supplemented with 0.1% BSA, 0.1 m*M* ascorbic acid, 2.5×10^{-5} *M* bacitracin, 5 μg/ml human insulin, 20 μg/ml human transferrin, 10^{-7} *M* putrescine, 3×10^{-8} *M* selenite, 100 μg/ml streptomycin (Sigma Chemical Co.), 100 units/ml penicillin, 2 m*M* L-glutamine, and 0.3 μg/ml fungizone. A typical experiment was performed after a total of 4 days in culture. The cells were then washed five times with the serum free, defined D-MEM, 1.0 ml of media was added to each well, and the cells allowed to equilibrate at 37°C for 1 hr before experiments were begun. Each treatment was performed in duplicate on cells isolated from different sheep ($n = 5$ represents duplicate wells of cells isolated from five sheep). All secretagogues used in these experiments were dissolved directly in D-MEM-defined medium except bromocriptine which was solubilized in ethanol and subsequently diluted so that the final ethanol concentration was 0.01%. Ethanol concentrations of 0.1% or less had no effect on the cells' response to stimulation by GHRH ($hGHRH_{1-44}$; gift from Dr. M. Moseley, The Upjohn Company, Kalamazoo, MI) or inhibition of GHRH-stimulated GH release by SS (Penninsula Laboratories, Belmont, CA). All media samples were stored frozen at −20°C pending radioimmunoassay for ovine GH.

Initial experiments were performed to determine the time and dose of agonist to produce a maximal effect. The neurotransmitter agonists selected for study (for improved stability compared with the neurotransmitters) were the dopaminergic agonist, bromocriptine (BRO, 10^{-6} *M;* Sigma Chemical Co.), the α_2-adrenergic agonist, clonidine (CLON, 10^{-6} *M;* Sigma Chemical Co.), the serotonergic agonist, quipazine (QUIP, 10^{-6} *M;* Research Biochemicals, Inc., Natcik, MO), the α_1-adrenergic agonist, methoxamine (METH, 10^{-6} *M;* Sigma Chemical Co.), the β-adrenergic agonist, isoproterenol (ISO, 10^{-6} *M;* Sigma Chemical Co.). These receptor agonists offer more specificity for receptor subtypes and are generally more stable in culture than the neurotransmitters.

Neurotransmitter receptor agonists (at doses selected from the dose response study) were then examined to determine if selected receptor antagonists could block receptor activation by the agonists. The antagonists were propranolol (10^{-6} *M,* for ISO; Research Biochemicals Inc.), benoxathian (10^{-6} *M,* for METH; Research Biochemicals Inc.), phenoxybenzamine (10^{-6} *M,* for CLON; Research Biochemicals Inc.), and haloperidol (10^{-6} *M,* for BRO; Sigma Chemical Co.). Antagonists were added 10 min prior to addition of the agonists. The incubation time was based on the time-course study and varied between neurotransmitter agonists. At the end of the experiment, the media was removed and stored frozen for later GH assay.

A study was then designed to determine the effects of neurotransmitter receptor agonists on GHRH actions. Cells were incubated in the presence of GHRH (10^{-10} *M* or 10^{-8} *M*) plus the receptor agonist at the dose and duration previously determined. In addition, SS (10^{-7} *M*) was used as a known inhibitor of GHRH-stimulated GH release.

The effects of neurotransmitter agonists on basal and GHRH (10^{-8} *M*)-stimulated GH release and

mRNA content were determined. Cells (10^6 per well) were treated with either GRH, SS, or neurotransmitter agonists or GHRH plus SS, or GHRH plus neurotransmitter receptor agonist at doses determined previously. Samples (50 μl) of media were removed at 0.5, 1, 2, 4, 6, 12, 24, and 48 hr. An equivalent volume of media containing appropriate treatments was used to maintain volume. After 24 or 48 hr, cells were washed three times in cold phosphate buffered saline (pH 7.4) and extracted for total mRNA (see below).

Radioimmunoassay. Growth hormone concentrations in culture medium were assayed by using antisera oGH-2 (National Pituitary Agency, Baltimore, MD) and oGH-I-1 (USDA, Beltsville, MD) for the assay standards. The assay was previously validated for oGH (15, 17). The presence of tissue culture medium was accounted for by addition of the same volume (10 μl) of medium to the standards as was present in the samples. Dilution of incubation media containing GH produced a displacement curve parallel to the standard curve. Recovery of GH added to incubation medium was 102.8% ± 2.7% and the intra- and interassay coefficients of variation were 2.8% and 9.0%, respectively.

Extraction and Measurement of RNA. Total cellular RNA was extracted after 24 or 48 hr of culture using the RNAzol method (Cinna/Biotecx Laboratories International, Inc., Friendwood, TX), a modification of the method of Chomczynski and Sacchi (18). The purity of the RNA preparation was determined by measuring the A260/A280 ratio, which was 1.8–2.1 for all preparations. Yield of total RNA was 15–20 μg total RNA/10^6 cells in a 35-mm well.

Northern blots were performed on 4 μg of total RNA as described (19) using a 1.2% agarose-formaldehyde gel. The RNA in the gel was transferred by capillary action to nitrocellulose filters (Schleicher and Schuel, Keene, NH). After 8-hr prehybridization, the blots were hybridized in a buffer of 50% formamide, 2 × NaCl/cit/0.5% SDS (2 × SSC, 0.5% SDS), 5 × Dernhardt's solution, 50 m*M* sodium phosphate, sonicated denatured salmon sperm DNA (200 μg/ml), 400 μg/ml yeast RNA, 10 m*M* Tris (pH 7.0) with nick translated, (α^{32}P)dCTP (Amersham, Arlington Heights, IL) labeled cDNA probe for bovine GH (830 bp; gift from Dr. F. Rottman, Case Western Reserve University, Cleveland, OH) with a specific activity of $\geq 1 \times 10^8$ CPM/μg. Blots were subsequently reprobed with α-actin cDNA (gift from Dr. Haron Roche, Institute of Molecular Biology, Nutley, NJ). The hybridizations were carried out for 40 hr at 42°C. After hybridization, nitrocellulose filters were washed with three changes of 2 × SSC, 0.5% SDS for 10 min at room temperature and two changes of 0.1 × SSC, 0.1% SDS for 10 min at room temperature. Autoradiograms were quantified by scanning with a computer controlled laser densitometer (EC Apparatus, St. Petersburg, FL).

Although a species homologous probe was not used, the bovine and ovine message is very similar (20). Aliquots of 2, 4, and 8 μg total RNA were scanned to insure that the signal was linear over the range of these experiments. Selected Northern blots (not for data collection) were subjected to three washes (15 min, room temperature in 2 × SSC, 0.1% SDS) and a higher stringency wash (65°C, 0.2 × SSC, 0.1% SDS for 30 min) to insure that the two bands represented specific hybridization signals. Finally, the insert was removed from the plasmid to insure that the hybridization was to the bGH cDNA and not the plasmid.

Statistical Analysis. Analysis of variance which included specific comparisons of treatment means was performed on autoradiograms and radioimmunoassay data (SAS, Gary, NC). A probability level of less than 5% (0.05) was chosen for determining statistical differences.

Results

The dose-response study indicated that 10^{-8} *M* GHRH and 10^{-6} *M* for the neurotransmitter receptor agonists produced a maximal response (Fig. 1). Exposure to GRH or neurotransmitter agonists produced maximal effects at durations as follows: GRH, 3 hr; BRO, 2 hr; CLON, 4 hr; and METH and ISO, 90 min (data not shown).

The ability of selected neurotransmitter antagonists to block the actions of the agonists used are presented in Table I. In each case the antagonist blunted the effects of the respective agonist. Haloperidol (10^{-6} *M*) was able to antagonize the BRO inhibitory effect (Table I) on basal GH release at 4 hr incubation. Similarly, CLON inhibition of GH secretion was antagonized by 10^{-6} *M* PHE (Table I). The stimulatory effect of ISO and METH on GH secretion ($P < 0.05$) were blocked by pretreatment with 10^{-6} *M* propranolol and 10^{-6} *M* benoxathian (BEN), respectively (Table I), at 90 min of incubation.

Somatostatin (10^{-7} *M*) significantly ($P < 0.01$) inhibited (75%, 70%) 10^{-10} *M* and 10^{-8} *M* GHRH-stimulated GH release, respectively (Fig. 2). However, SS had no effect on basal (control) GH concentrations. Although SS inhibited GHRH-stimulated GH release, the GH release was still higher ($P < 0.01$) than the control. The amount of GH secreted in the SS plus 10^{-10} *M* GHRH-treated group was lower ($P < 0.05$) than SS plus 10^{-8} *M* GHRH (Fig. 2). Bromocriptine caused a marked ($P < 0.01$) inhibition of basal and GHRH-stimulated GH release (Fig. 2). The release of GH in response to BRO (10^{-6} *M*) plus GHRH (10^{-10} *M*, 10^{-8} *M*) were 62% and 57% lower than the release by GHRH alone ($P < 0.01$). However, the effect of

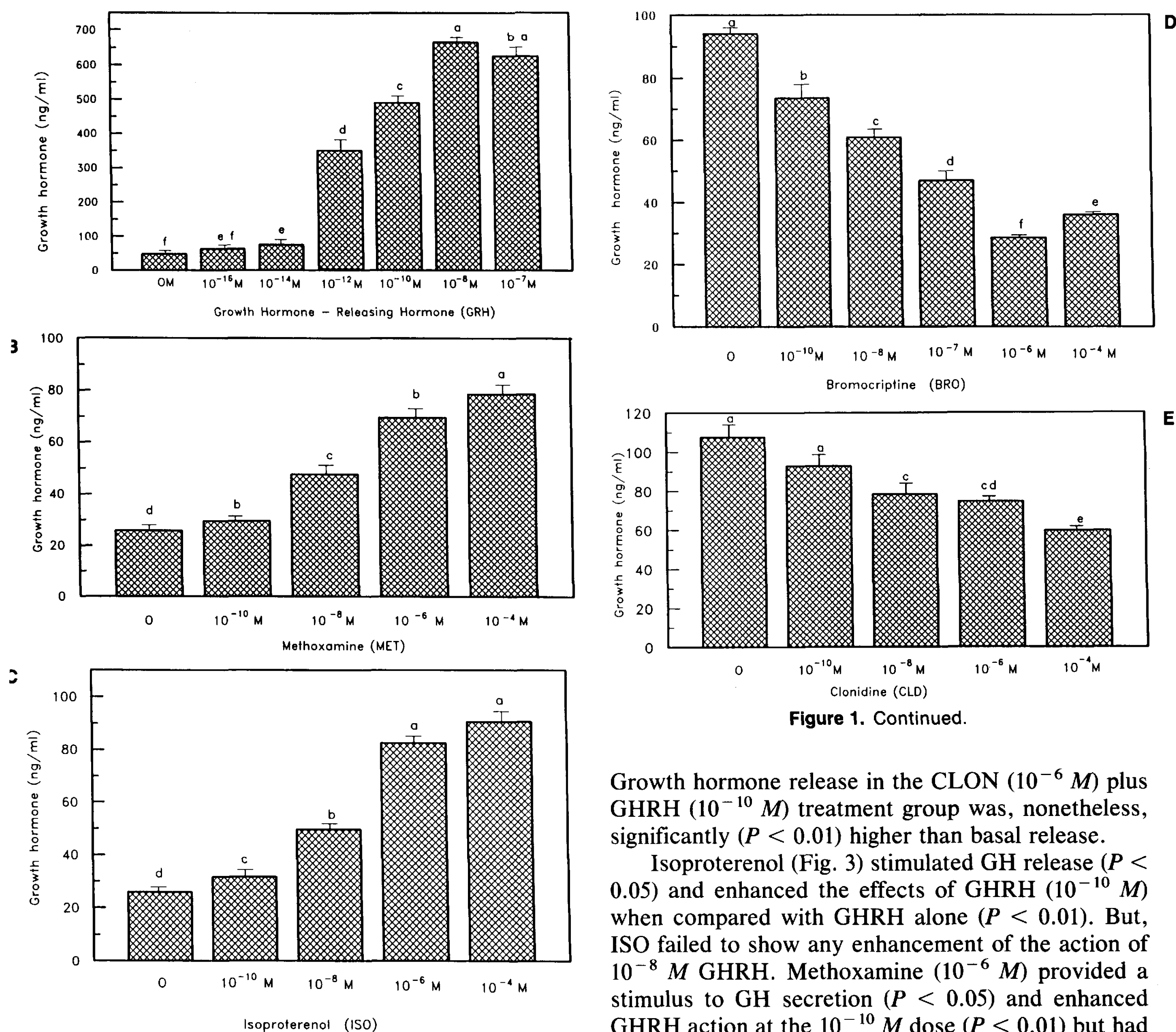


Figure 1a–1e. Dose-Response effects of neurotransmitters on GH release from cultured ovine pituitary cells. Different letters within each graph represent significant differences, like letters represent nonsignificant comparisons. Duration of incubations differed and were based on data in Table I. $P < 0.05$ was used to determine significance.

Figure 1. Continued.

BRO plus GHRH was still ($P < 0.01$) greater than the control, indicating incomplete inhibition. The BRO plus GHRH (10^{-8} *M*) treatment released more ($P < 0.05$) GH than BRO plus the lower concentration of GHRH (10^{-10} *M*). Clonidine (10^{-6} *M*) also lowered ($P < 0.05$) basal GH secretion (Fig. 2) while GH released in response to CLON (10^{-6} *M*) plus GHRH (10^{-10} *M*) produced a 42% inhibition of GH release into the media, which was different ($P < 0.01$) from the GHRH (10^{-10} *M*)-treated groups. However CLON failed to show any inhibition at the 10^{-8} *M* dose of GHRH. Growth hormone release in the CLON (10^{-6} *M*) plus GHRH (10^{-10} *M*) treatment group was, nonetheless, significantly ($P < 0.01$) higher than basal release.

Isoproterenol (Fig. 3) stimulated GH release ($P < 0.05$) and enhanced the effects of GHRH (10^{-10} *M*) when compared with GHRH alone ($P < 0.01$). But, ISO failed to show any enhancement of the action of 10^{-8} *M* GHRH. Methoxamine (10^{-6} *M*) provided a stimulus to GH secretion ($P < 0.05$) and enhanced GHRH action at the 10^{-10} *M* dose ($P < 0.01$) but had no effect on 10^{-8} *M* GHRH-stimulated GH release (Fig. 3).

The effects of neurotransmitter receptor agonists on GH messenger ribonucleic acid (mRNA) content in sheep anterior pituitary cells in culture for 24 and 48 hr incubation periods are presented in Table II. Two different species of GH mRNA were identified in total RNA extracts (Fig. 4). The two species, designated SP1 and SP2, had sizes of 1.1 and 3.2 kb, respectively. Both messages were increased ($P < 0.01$) by treatment with GHRH for 24 or 48 hr. Methoxamine caused a significant ($P < 0.05$) increase in SP1 GH mRNA, at 24 and 48 hr, whereas isoproterenol only increased SP1 significantly ($P < 0.05$) after 48-hr incubation. Bromocriptine increased ($P < 0.05$) the content of both species of GH mRNA after treatment for 24 or 48 hr. Neither QUIP or CLON produced any effect on mRNA content. The effects of BRO, ISO, CLON,

Table I. The Effect of Neurotransmitter Receptor Antagonists on Neurotransmitter-Stimulated Growth Hormone Secretion

Treatment	Dose	Growth hormone (ng/ml)			
		Control	Antagonist	Agonist	Agon + antag
Clonidine (Phenoxybenzamine)	(10^{-6} *M*)	83.1 ± 5.2	87.6 ± 7.1	67.5 ± 2.2*	90.5 ± 7.9
Bromocriptine (Haloperidol)	(10^{-6} *M*)	82.1 ± 5.1	83.0 ± 6.8	50.8 ± 4.6*	78.7 ± 6.5
Methoxamine (Benoxathian)	(10^{-6} *M*)	32.5 ± 2.6	33.0 ± 3.7	72.5 ± 7.1*	37.2 ± 5.1
Isoproterenol (Propranolol)	(10^{-6} *M*)	33.0 ± 5.2	33.8 ± 6.9	84.7 ± 3.5*	32.2 ± 6.5

Note. Receptor agonists are listed first and the respective antagonist follows in parentheses. The dose of antagonist is the same as for the agonist. Incubation times for CLON and BRO were 4 hr while incubation times for MET and ISO were 90 min. $n = 5$.
* Values differ from respective controls, $P < 0.05$.

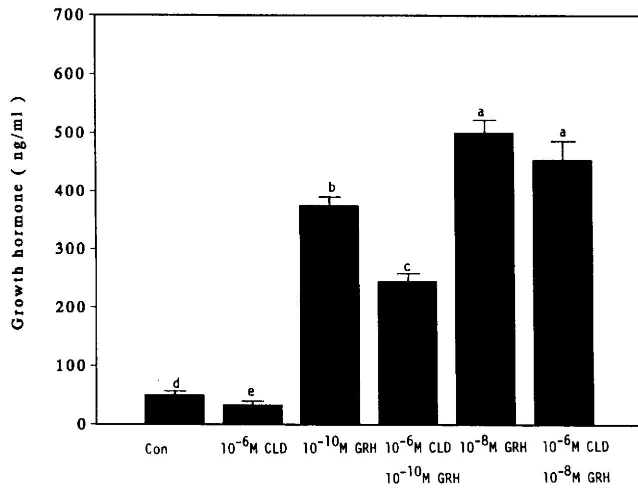
METH, and SS on GHRH (10^{-6} *M*)-stimulated total cellular mRNA was determined (Table III). The GHRH-treated group had a 2.0- to 2.5-fold ($P < 0.05$) increase in GH mRNA (SP1 and SP2) content. Treatment with BRO enhanced ($P < 0.05$) basal GH-mRNA (SP1 and SP2). In the group treated with BRO plus GHRH, only SP1 GH mRNA content was increased ($P < 0.05$) over the GHRH-treated group. Isoproterenol and CLON-treated groups showed no enhancing or inhibiting effect on any species of GH mRNA (Table III). Their combination with GHRH also failed to show any differences on mRNA content from that of GHRH. Methoxamine enhanced only the SP1 GH mRNA above the control content ($P < 0.05$). The combination of METH and GHRH showed an increase in SP1 GH mRNA, which was greater ($P < 0.05$) than GHRH-stimulated SP1 GH mRNA. Somatostatin (SS) had no effect on basal GH mRNA content, but in combination with GHRH, SS blocked GHRH effects to increase GH mRNA content (Table III).

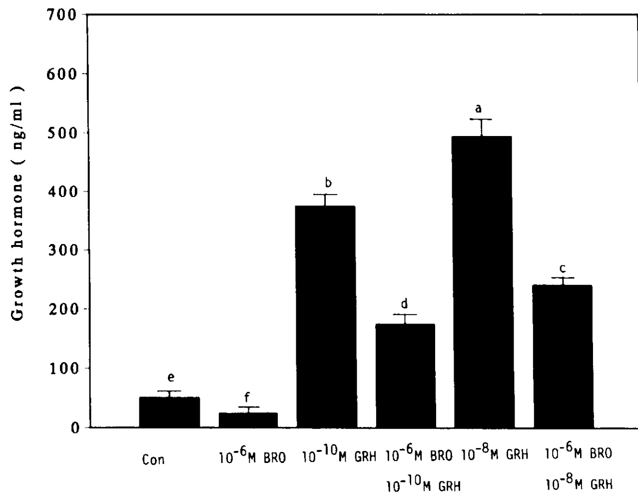
Discussion

Examination of Northern blots of total ovine pituitary mRNA revealed the presence of two transcripts, sized at 1.1 and 3.2 kb. Both bands were present after a high stringency wash, were present when either the plasmid or the GH cDNA insert were used in the hybridization, and increased in quantity in response to GHRH. This suggests the bands represent specific hybridization of the bGH cDNA to the GH mRNA. Data from other laboratories have presented Northern blots of total RNA probed with an ovine GH cDNA which revealed bands of 0.8–1.0 kb, a second band between the 18 and 28s ribosomal bands (corresponding to our 3.2 kb band), and an occasional larger band (21). In the rat, nuclear precursors have been reported which are larger than the mature GH mRNA (23). Nuclear precursors of 6.7, 5.6, 2.3, and 1.0 kb have been found in GC cells (22), as well as a high molecular weight variant of GH in bovine pituitary (23). Whether the 3.2 kb form represents an independent transcript, a splice variant or a precursor which is subsequently spliced to the 1.1 kb form was beyond the scope of the present study.

The effects of GHRH are to increase secretion of GH and to increase GH gene expression. These data are in agreement with the expected results from studies of rats, cattle, and sheep (24–26). The primary inhibition of GH release and gene expression is provided via SS. Data in this study were generally in agreement, however, SS produced no inhibition of basal GH release in this study. This is consistent with the *in vivo* effects of SS on basal GH concentrations but differs for data in neonatal sheep (26) and in rats (24). Unlike data in cattle, where there was a slight inhibition of GHRH-stimulated GH mRNA accumulation (25), SS prevented the GHRH-stimulated rise in GH mRNA content in sheep pituitary cells. In rats, basal GH release and basal GH mRNA accumulation were inhibited by SS (24), whereas in sheep pituitary cells in this study, there was no effect of SS on basal GH mRNA content.

While dopaminergic receptor activation effects on GH release have been unclear in some species, data in the sheep demonstrate a clear inhibition of GH release *in vitro* (7, 8). Activation of dopaminergic receptors by bromocriptine produced a potent inhibition of both basal and GHRH-stimulated GH release. Activation of pituitary dopaminergic receptors has also been shown to inhibit forskolin (8) and TRH (7)-stimulated GH release in either cultured sheep pituitary cells (8) or pituitary explants (7). Although the mechanism for the inhibition is unclear, BRO will inhibit cyclic AMP accumulation in sheep pituitary cells, though the mechanism appears not to involve a G_i protein-dependent mechanism (27). Based on these studies demonstrating inhibition of GH release, an inhibition of GH mRNA accumulation was expected. The results, however, in-





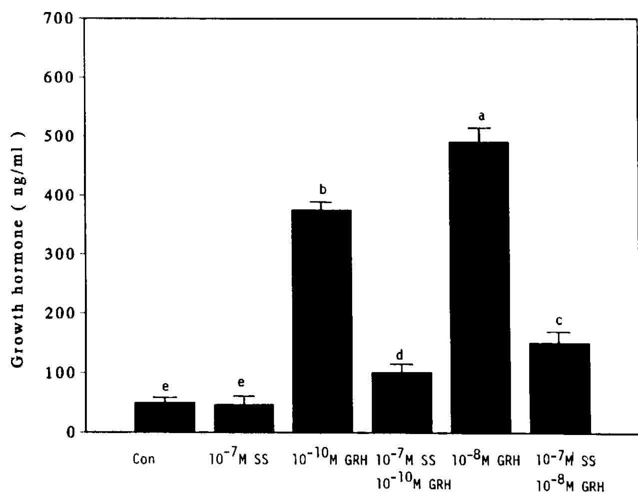
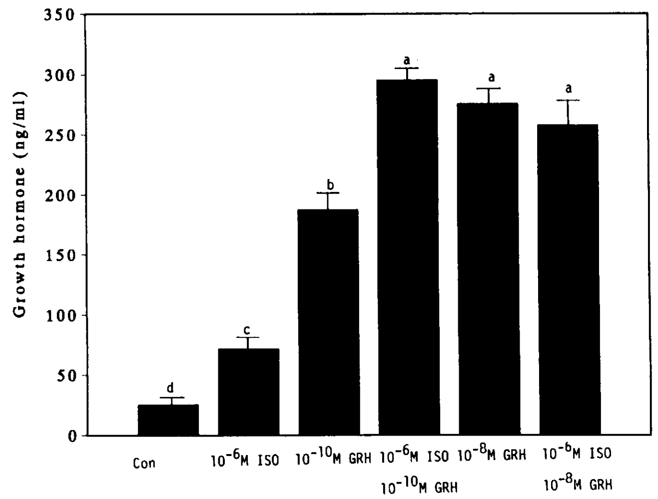


Figure 2. Effect of inhibitory neurotransmitters and somatostatin on GHRH-stimulated GH release from cultured ovine pituitary cells. Different letters indicate significant differences.

dicated either an increase in GH mRNA production or a decrease in GH mRNA turnover in the presence of BRO. One possible mechanism to account for the disparate results between the effects of BRO on GH release and mRNA content is the presence of two



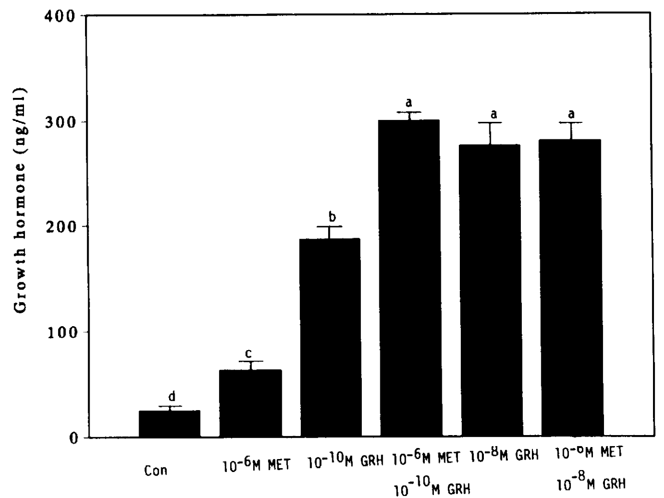


Figure 3. Interaction of stimulatory neurotransmitters on GH release from cultured ovine pituitary cells. Different letters indicate significant differences.

classes of dopaminergic receptor (28), a D_1 (stimulatory) and a D_2 (inhibitory) receptor, which may independently regulate GH release and gene expression. Based on these data and others (7, 8), as well as the availability of dopamine to the somatotrope, dopamine

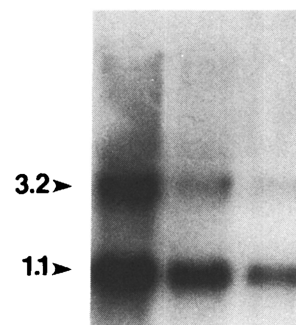


Figure 4. Northern blot of total ovine pituitary mRNA. Lanes from left to right were loaded with 8, 4 and 2 μg total mRNA. Numbers at left indicate size (kb).

Table II. The Effect of GHRH, Methoxamine, Isoproterenol, Clonidine, Quipazine, and Bromocriptine on GH mRNA Content

Treatment	Time 24 hr		Time 48 hr	
	SP1	SP2	SP1	SP2
Control	100.00	100.00	100.00	100.00
10^{-8} *M* GHRH	245.30 ± 31.06**	190.86 ± 10.87**	188.69 ± 14.50**	198.72 ± 5.00**
10^{-6} *M* Methoxamine	148.80 ± 7.12*	107.11 ± 3.50	157.84 ± 6.87*	109.68 ± 7.50
10^{-6} *M* Isoproterenol	77.00 ± 16.67	106.37 ± 4.50	146.46 ± 7.50*	114.56 ± 2.50
10^{-6} *M* Quipazine	99.50 ± 5.10	101.50 ± 2.50	105.60 ± 3.50	98.58 ± 2.50
10^{-6} *M* Clonidine	113.95 ± 12.71	100.69 ± 2.50	120.51 ± 5.50	110.46 ± 5.80
10^{-6} *M* Bromocriptine	164.87 ± 11.21*	193.55 ± 14.51**	155.40 ± 7.50*	163.64 ± 11.78*

Note. The cultured pituitary cells were treated on Day 4 and the cells were extracted at 24 or 48 hr for total cellular RNA. Two different species, denoted as SP1 and SP2, of total GH mRNA were present, which are 1.1 and 3.2 kilobases in size, respectively. The control is taken as a reference point (100.00%) to which other treatments were compared. GH mRNA was corrected to actin mRNA. Values are the mean ± SEM (5 independent experiments) of autoradiograms. Asterisks (*, **) indicate that the value is significantly ($P < 0.05$; 0.01) different from control at that incubation time.

Table III. Effect of Neurotransmitter Receptor Agonists on Basal and Growth Hormone-Releasing Hormone-Stimulated Growth Hormone Messenger RNA Content

Treatment	Without GHRH		With GHRH (10^{-8} *M*)	
	SP1	SP2	SP1	SP2
Control	100.0^{d}	100.0^{d}	268.8 ± 12.1^{c}	204.5 ± 6.8^{b}
BRO (10^{-6} *M*)	155.4 ± 10.5^{c}	178.5 ± 10.8^{b}	300.5 ± 9.3^{b}	245.5 ± 16.5^{b}
ISO (10^{-6} *M*)	98.5 ± 5.2^{d}	118.5 ± 8.5^{d}	$275.1 \pm 10.5^{c,b}$	215.5 ± 7.8^{b}
CLON (10^{-6} *M*)	102.9 ± 6.9^{d}	96.6 ± 3.5^{d}	256.6 ± 10.5^{c}	203.1 ± 3.7^{b}
MET (10^{-6} *M*)	141.9 ± 8.5^{c}	100.0 ± 4.6^{d}	296.3 ± 11.5^{b}	221.8 ± 10.9^{b}
SS (10^{-7} *M*)	100.4 ± 0.7^{d}	100.5 ± 4.1^{d}	102.3 ± 5.1^{d}	104.9 ± 2.0^{d}

Note. Cultured pituitary cells were treated on Day 4 of culture and were extracted for total RNA after 24-hr treatment with the indicated neurotransmitter agonist in the presence or absence of GHRH. SP1 represents 1.1 kb GH mRNA while SP2 is a 3.2 kb mRNA. Controls were set to 100.0%, with which other treatments were compared. GH mRNA was corrected to actin mRNA. Values are the mean of duplicate samples for five independent replications. Within columns, means with the same superscript are not different compared with controls.

could regulate basal and GHRH-stimulated GH release in the sheep without compromising GH synthesis. Indeed, dopamine is considered a tonic regulator of prolactin release (6).

Our data in the sheep are consistent with α_2-adrenergic receptors mediating inhibitory actions of adrenergic agonists on pituitary GH release, while α_1- and β-adrenergic receptors appear to mediate the stimulatory actions of adrenergic agonists on GH release. Similar studies in rats also indicate a stimulation of GH release by α_1- and β-adrenergic receptors (29). Although all three receptor classes were capable of altering GH release, only METH (α_1) had an effect on GH mRNA accumulation. The effects of METH and GHRH on GH mRNA accumulation were additive, suggesting use of the same mechanism for increasing GH mRNA accumulation, most likely a protein kinase A-dependent mechanism (8). Determination of whether stimulatory or inhibitory actions of adrenergic receptor activation predominate at the somatotrope were beyond the scope of this study, however, extrapolation of sheep pituitary explant data (7), where norepinephrine inhibited TRH-stimulated GH release, would suggest the α_2 effect may be the predominant receptor effect at the sheep pituitary. Similarly, iv epinephrine administered to sheep inhibited GH release, an effect reversed by phentolamine but not propranolol (30).

One difficulty with these data is that the prolonged incubation conditions create a situation where GH is present in increasing concentration over time. Since GH has a negative feedback effect on GH regulatory mechanisms, the neurotransmitter agonists may have had their effects blunted, especially in the gene expression studies. In addition, cell dispersal and culture conditions may influence receptor expression. In spite of these cautions, the data on secretion are consistent with that found in other species and the effects on GH mRNA content suggest the value of additional studies on regulation of GH gene transcription and message turnover.

In summary, these data support and extend previous observations suggesting a role for neurotransmitters in regulating GH secretion and indicate a pituitary site of action in the sheep. Further, the results provide evidence that mRNA content is regulated by neuro-

transmitters, hence, not only GH secretion but also synthesis may be regulated by neurotransmitters.

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