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# Comparison of the effect of hypothalamic neuropeptides upon luteinizing hormone secretion by cultured rat anterior pituitary cells

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*Bovine median eminence contains a factor different from gonadotropin-releasing hormone (GnRH) that increases basal luteinizing hormone (LH) secretion and potentiates GnRH-stimulated LH release. We compared the effects of hypothalamic neuropeptides on basal and GnRH-stimulated LH secretion using rat pituitary cells under static incubation conditions to determine if any of them mimics the LH-releasing activity not attributable to GnRH present in bovine median eminence extracts. Both, galanin and neurotensin ($10^{-9}$-$10^{-5}$ M) stimulated basal LH secretion in a dose-response manner. Galanin increased 3-4 fold and neurotensin doubled the basal LH secretion. The GnRH antagonist Nal-Glu $10^{-6}$ M abolished the effect of $10^{-7}$ M GnRH and $10^{-5}$ M neurotensin, but did not block the LH-releasing activity of galanin. Leucine-enkephalin, ß-endorphin, substance P and neuropeptide Y (NPY) did not alter basal LH secretion. Neuropeptides produced three types of response on GnRH-stimulated LH release. First, leucine-enkephalin and ß-endorphin ($10^{-9}$-$10^{-5}$ M) showed a dose-dependent inhibition of GnRH-stimulated LH release. At $10^{-5}$ M the inhibition was complete with leucine-enkephalin and only 30% with ß-endorphin. Both were blocked by naloxone. Second, substance P showed an inverted U type response on GnRH-stimulated LH secretion. At $10^{-9}$ M this peptide potentiated the action of GnRH. This effect decreased when the dose of substance P was increased to $10^{-7}$ M and turned inhibitory at $10^{-5}$ M when $10^{-7}$ M GnRH was used. Third, galanin and NPY potentiated the effect of GnRH on LH secretion. Neurotensin had no effect on GnRH-stimulated LH release. In conclusion, rat gonadotrophs present diverse responses to neuropeptides at physiological concentrations, and -apart from GnRH- galanin is most likely the other factor present in bovine median eminence extracts that stimulates LH secretion. The data lend further support to a role of galanin in the control of LH secretion.*

**Key words:** *luteinizing hormone, neuropeptides, opioid peptides, pituitary.*

## INTRODUCTION

It is well established that pituitary gonadotropin secretion is primarily under the control of gonadotropin-releasing hormone (GnRH), but there is increasing evidence that this process is modulated also by multiple other factors of gonadal and hypothalamic origin. In previous studies (22), we found that bovine median eminence (ME) extracts increased both basal and GnRH-stimulated luteinizing hormone (LH) secretion from dispersed pituitary cell cultures. This LH-releasing activity was not entirely attributed to

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GnRH because it differed in its immunoreactivity, it could stimulate LH release when GnRH receptors were blocked and the maximal response obtained was larger than that evoked by GnRH alone. To approach the identification of this factor, it was deemed appropriate to determine first whether or not neuropeptides known to be present in the hypothalamus, such as galanin (27), neurotensin (1, 28), neuropeptide Y (NPY) (30) and opioid peptides (19), could account for this activity. Although, opiates (5,7,19), corticotrophin-releasing hormone (CRH) (6), α-melanocyte-stimulating hormone (α-MSH) (29), galanin (24), NPY (18), pituitary adenylate cyclase-activating polypeptide (PACAP) (16) and substance P (3,43) have been shown to alter LH release, a detailed comparison of their effects on LH secretion with that of ME extracts is not warranted from the data reported, because they were obtained under widely different experimental conditions.

Here we present a systematic comparison of the effects of various neuropeptides upon basal and GnRH-evoked LH secretion utilizing the same assay system used for the ME extracts. Dispersed rat pituitary cells in culture under static incubation conditions were used to evaluate the LH-releasing activity of the neuropeptides, their interactions with GnRH and whether the effects elicited by concentrations used compared to those attained at portal blood and/or pituitary itself.

## MATERIALS AND METHODS

### *Reagents*

GnRH was obtained from Bachem Bioscience Inc. The GnRH antagonist [Ac-D2Nal[1], D4ClPhe[2], D3Pal[3], Arg[5], D-4-p-methoxybenzoyl-2-amino butyric acid[6], DAla[10]]-GnRH (Nal-Glu) was a gift from Dr Jean Rivier, Salk Institute, La Jolla, California. All other peptides, tissue culture media and reagents were obtained from Sigma Chem. Co.

### *Culture of rat anterior pituitary cells*

Anterior pituitary glands obtained from 60-days old female Sprague-Dawley rats were

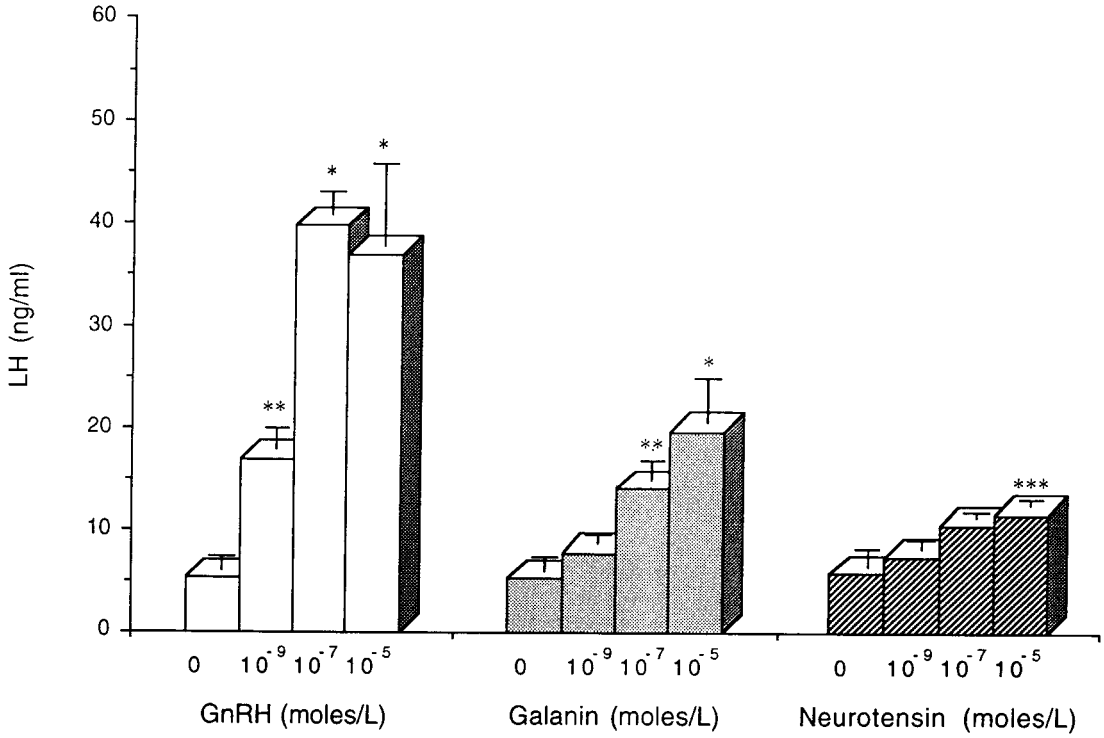


**Fig 1.** *Effects of GnRH, galanin and neurotensin on LH release by cultured rat pituitary cells.* Cells were cultured for 3 days in 96-well plates (1.5 x $10^5$ cells per well) before incubation with increasing doses of GnRH, galanin or neurotensin for 2 h at 37°C. Media were collected at the end of the experiment and their LH contents determined by ELISA. Bars represent the means ± SD's of 8 replicates. * $p < 0.001$, ** $p < 0.005$ and *** $p < 0.01$ *vs* respective control (0).

dispersed as described previously (22), to obtain primary cell cultures. Dispersed cells were plated at a density of 1.5 x $10^5$ cells per well in 96-well plates (FALCON 3872 PRIMARIA) and cultured at 37°C in an atmosphere of 95% air-5% $CO_2$ for three days.

*LH secretion experiments*

The cells were washed twice with serum-free medium 199 (without phenol red) buffered with 10 mM HEPES, pH 7.4; containing 0.3% bovine seroalbumin (M199-BSA). The cells were then incubated for 2h at 37° C in M199-BSA alone (basal secretion) or M199-BSA containing test substances. After incubation, the media were collected for measurement of LH by ELISA. All assays were performed in eight replicates at a final volume of 0.3 ml. The effects of test substances were examined in at least two individual experiments.

*LH-ELISA*

A double sandwich ELISA system described by Leiva and de la Lastra (21), was carried out in 96-well microtiter plates (Dynatech Lab. Inc.). Rat LH-RP-3 was used as standard and anti-bLH ß-subunit (monoclonal antibodies, clone 518 B7 obtained from Dr Jan Roser, Department of Animal Sciences, University of California, Davis, CA.), anti-rLH-S-10 (rabbit IgG, NIDDK) and anti-rabbit IgG peroxidase conjugate (Sigma Chem Co), were used as first, second and third antibody, respectively. All samples were assayed in duplicate. Intra-assay coefficients of variation for culture medium pools containing low, medium and high LH concentrations were 4.5, 2.3 and 6.9%, respectively. The inter-assay coefficients of variation for the same pools were 9.6, 7.3 and 8.5%, respectively.

*Statistics*

Values are presented as means ± standard deviations. Significant differences were evaluated by analysis of variance (46).

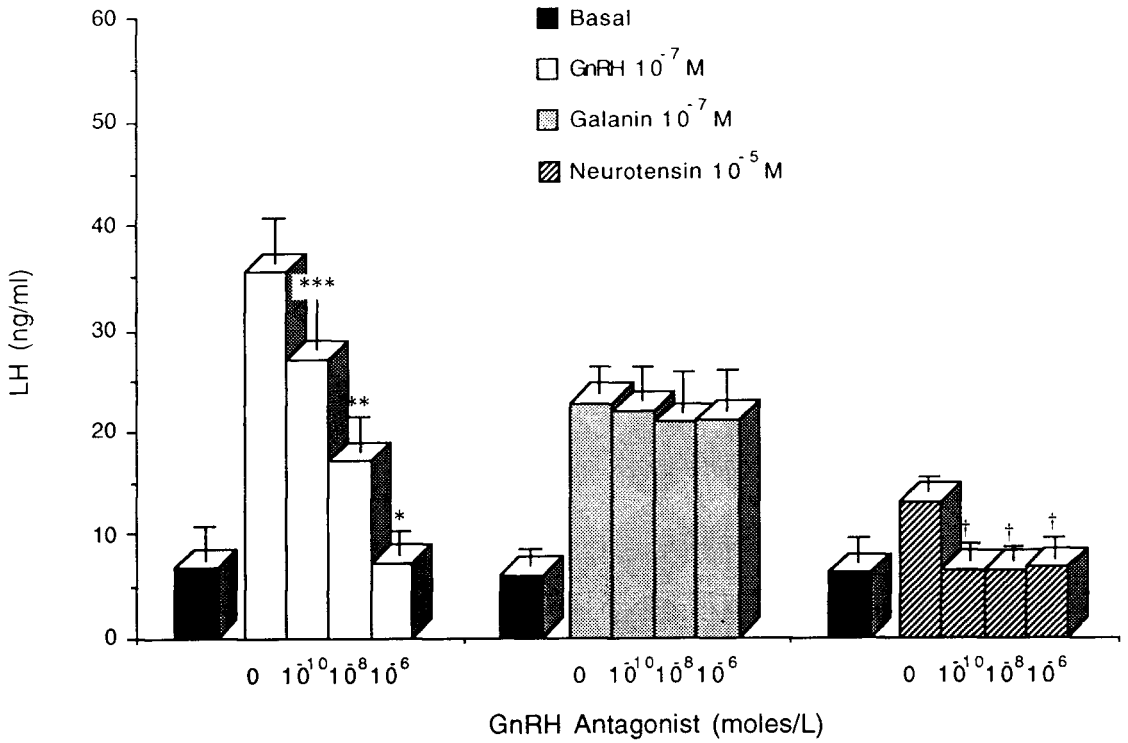


**Fig 2.** *Effects of the GnRH antagonist, Nal-Glu, on LH-releasing activity of GnRH, galanin and neurotensin.* After 3 days in culture, rat pituitary cells (1.5 x $10^5$ cells/well) were preincubated in the presence of increasing doses of Nal-Glu for 30 min and then were coincubated with GnRH $10^{-7}$ M, galanin $10^{-7}$ M or neurotensin $10^{-5}$ M, for 2 h at 37° C. LH in the media was determined by ELISA. Bars represent the means ± SD's of 8 replicates. * $p < 0.001$, ** $p < 0.005$ and *** $p < 0.01$ *vs* GnRH alone. † $p < 0.01$ *vs* neurotensin alone. Black bars represent basal LH secretion.

RESULTS

*Effect of neuropeptides on basal LH release*

Basal LH secretion from pituitary cells in the presence or absence of different neuropeptides (including GnRH) was assayed *in vitro*. Aside GnRH, galanin and neurotensin increased basal LH secretion (Fig 1). The effect of galanin ($10^{-9}$ M to $10^{-5}$ M) was clearly dose-dependent. The maximal effect elicited by galanin was obtained with the $10^{-5}$ M concentration and was a 3-4 fold increase over the basal secretion ($p < 0.001$). The effect of neurotensin was also dose-dependent and the maximal effect observed was a two fold increase ($p < 0.01$). The maximal effects of galanin and neurotensin $10^{-5}$ M represent about 50% and 25-30%, respectively, of the maximal LH release induced by GnRH. Opioid peptides (leucine-enkephalin, methionine-enkephaline, β-endorphin and dynorphin), substance P, neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP) did not significantly alter the basal LH secretion (data not shown).

The neuropeptides that stimulated basal LH secretion were tested on pituitary cells pretreated with the GnRH antagonist Nal-Glu (Fig 2). Nal-Glu $10^{-6}$ M fully suppressed the LH releasing activity of $10^{-7}$ M GnRH ($p < 0.001$) or $10^{-5}$ M neurotensin ($p < 0.01$), but did not affect the LH releasing activity of $10^{-7}$ M galanin.

*Effect of neuropeptides on GnRH-stimulated LH release*

Both leucine-enkephalin and ß-endorphin decreased the LH secretion evoked by $10^{-9}$ M and $10^{-7}$ M GnRH (Fig 3). Leucine-enkephalin ($10^{-9}$ M to $10^{-5}$ M) caused a dose-dependent decrease in LH secretion induced by $10^{-9}$ M and $10^{-7}$ M GnRH, leading to complete inhibition at $10^{-5}$ ($p < 0.001$). Beta-endorphin $10^{-5}$ M produced about 25% inhibition of LH release ($p < 0.05$). Figure 4 shows that naloxone prevented the inhibitory action

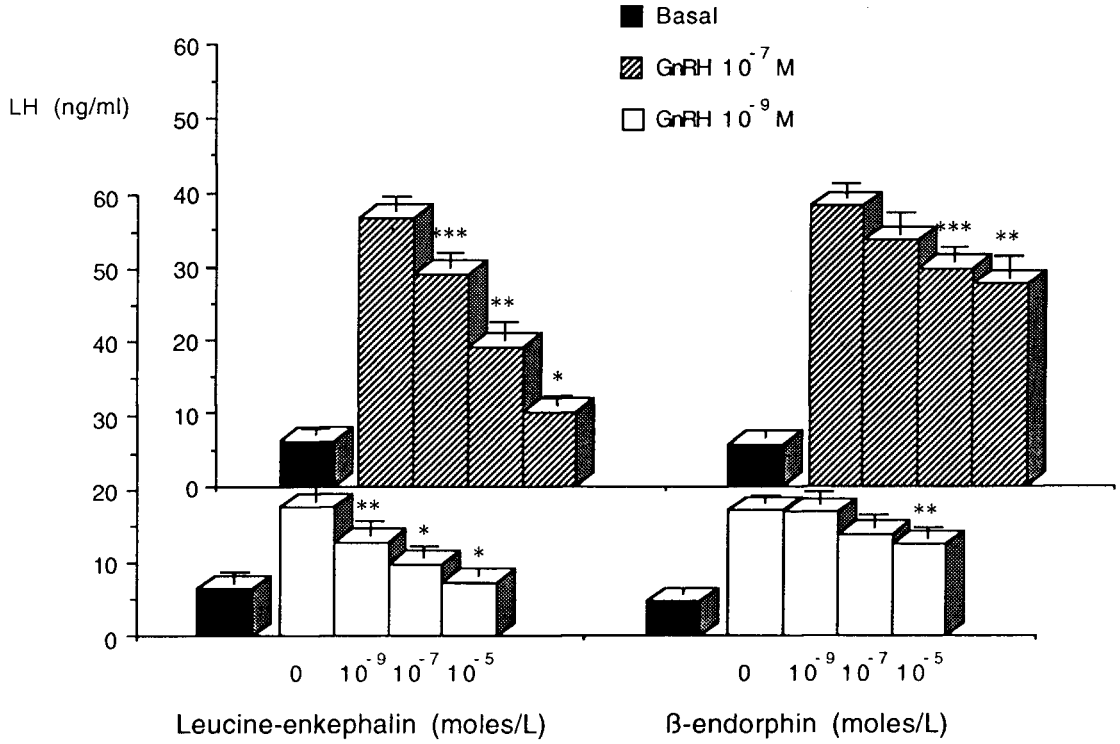


**Fig 3.** *Effects of leucine-enkephalin and ß-endorphin on GnRH-stimulated LH release.* After 3 days in culture, rat pituitary cells ($1.5 \times 10^5$ cells/well) were preincubated in the presence of increasing doses of leucine-enkephalin and ß-endorphin for 30 min and then were coincubated with GnRH $10^{-9}$ M or $10^{-7}$ M for 2 h at 37° C. Media were collected at the end of the experiment and their LH contents determined by ELISA. Bars represent the means ± SD's of 8 replicates. * $p < 0.001$, ** $p < 0.005$ and *** $p < 0.05$ *vs* GnRH alone. Black bars represent basal LH secretion.

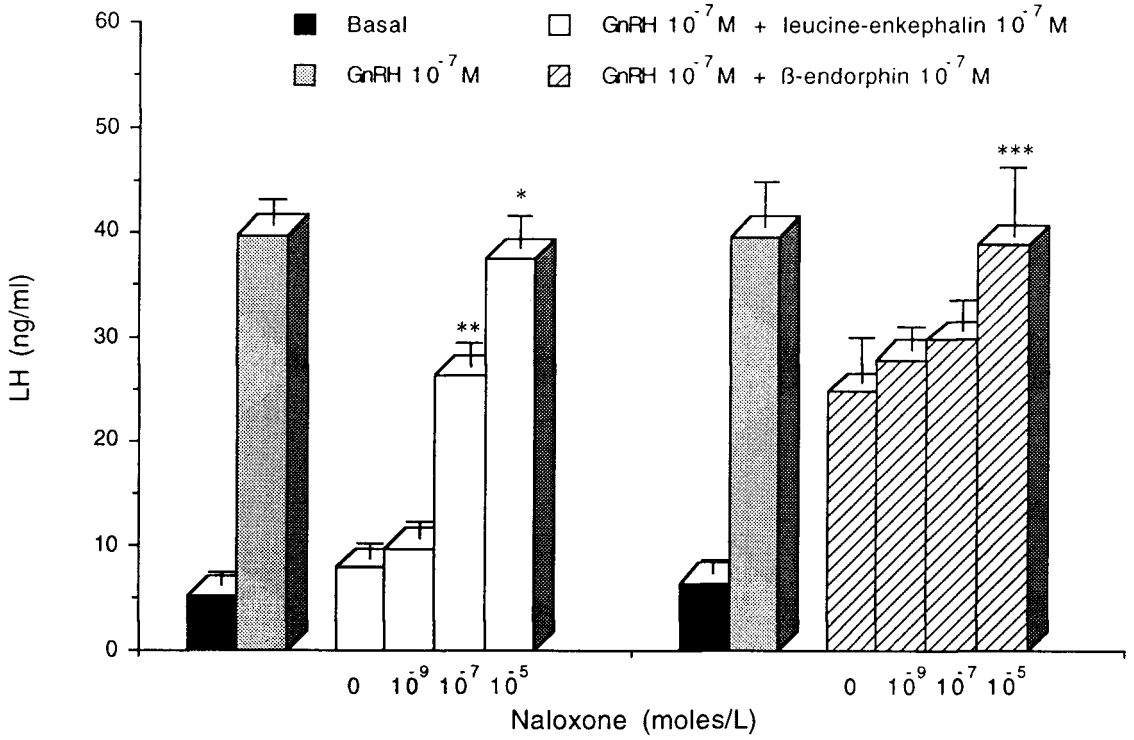


**Fig 4.** *Naloxone reverts the effect of opioid peptides (leucine-enkephalin and ß-endorphin) on GnRH-stimulated LH release.* Rat pituitary cells (1.5 x $10^5$ cells/well) were cultured for 3 days before the experiment. After 30 min of preincubation in the presence of increasing doses of naloxone, 10 µl of fresh medium alone or containing leucine-enkephalin or ß-endorphin was added and the preincubation continued for 30 min. At the end of the preincubation, cells were coincubated with GnRH $10^{-9}$ M or $10^{-7}$ M for 2 h at 37°C. LH in the medium was determined by ELISA. Bars represent the means ± SD's of 8 replicates. * $p < 0.001$, ** $p < 0.005$ and *** $p < 0.01$ *vs* respective control (0). Black bars represent basal LH secretion. Dashed bars represent GnRH-stimulated LH secretion.

of leucine-enkephalin and ß-endorphin on GnRH-stimulated LH release ($p < 0.001$ and 0.01, respectively).

Substance P showed an inverted U dose-response curve (Fig 5). A dose of $10^{-9}$ M substance P potentiated the action of GnRH $10^{-9}$ M ($p < 0.001$) or $10^{-7}$ M ($p < 0.005$). This effect decreased when the dose of substance P was increased to $10^{-7}$ M and turned inhibitory at $10^{-5}$ M when $10^{-7}$ M GnRH was used ($p < 0.05$). In contrast, neurotensin had no statistically significant effect on GnRH-stimulated LH release.

Galanin and NPY enhanced the effect of GnRH (Fig 6). NPY was apparently more effective than galanin in potentiating GnRH effect. Galanin $10^{-7}$ M and NPY $10^{-9}$ M doubled the LH released in response to $10^{-9}$ M GnRH ($p < 0.001$) and increased 1.5 times the LH release induced by GnRH $10^{-7}$ M ($p < 0.005$), respectively.

Vasoactive intestinal peptide, methionine-enkephaline and dynorphin had no effect on GnRH-stimulated LH release (data not shown).

## DISCUSSION

In previous studies (22), we demonstrated that bovine ME contains an LH-releasing activity not attributable to GnRH. Here, several neuropeptides known to be present in the hypothalamus were tested under the same conditions, to determine which one can account for this activity. Present results show that galanin mimics the effect of ME extracts on LH release since it increased basal as well as GnRH-stimulated LH secretion from rat pituitary cell cultures and was able to stimulate LH release when GnRH receptors were blocked. None of the other neuropeptides tested met all the above criteria, and galanin alone could account for the effects of ME extracts not attributable to GnRH. Furthermore, the maximal response to ME extracts

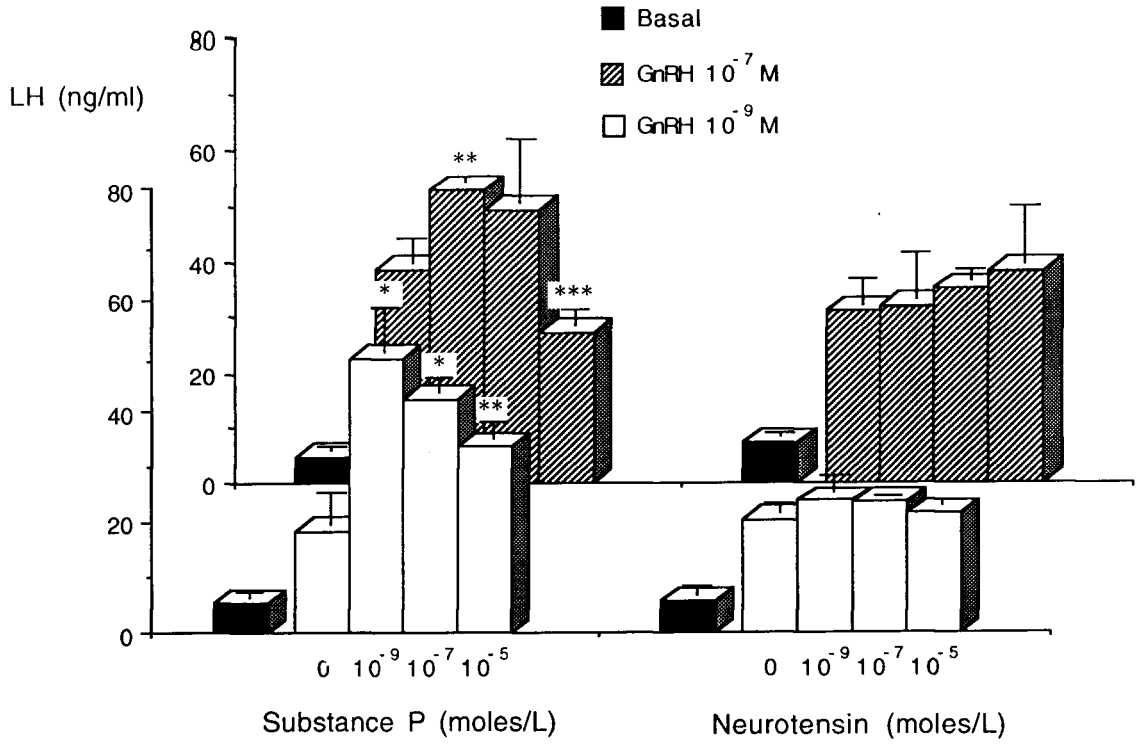


Fig 5. *Effects of substance P and neurotensin on GnRH-stimulated LH release.* After 3 days in culture, rat pituitary cells (1.5 x $10^5$ cells/well) were preincubated in the presence of increasing doses of substance P and neurotensin, for 30 min, and then were coincubated with GnRH $10^{-9}$ M or $10^{-7}$ M, for 2 h at 37° C. Media were collected at the end of the experiment and their LH contents determined by ELISA. Bars represent the means ± SD's of 8 replicates. * $p < 0.001$, ** $p < 0.005$ and *** $p < 0.05$ *vs* GnRH alone. Black bars represent basal LH secretion.

was larger than that to GnRH alone and this difference was suppressed by the GnRH antagonist Nal-Glu (22). Present results show synergism between GnRH and galanin, and unabated effect of the latter in the presence of Nal-Glu. Thus, maximal LH secretory response to ME extracts is best explained by the presence of both GnRH and galanine in the extracts.

Galanin receptors have not been characterized in the anterior pituitary gland. Our results with the GnRH antagonist, Nal-Glu, indicate that galanin effects on LH secretion are neither mediated by the GnRH receptor nor require its cooperation. The effect of neurotensin was peculiar in that it increased basal but not stimulated secretion. The action of neurotensin could involve GnRH receptors because it was blocked by Nal-Glu, but the participation of specific neurotensin receptors sensitive to Nal-Glu is not discarded. Nonetheless, the first interpretation explains better why it acts on basal and not in stimulated secretion. The effects of ß-endorphin and leucine-enkephalin were blocked by the opiate antagonist naloxone. This indicates that they modulate LH secretion through specific receptors for opioid peptides that have been found in pituitary gland membranes (19, 23). The effects of NPY and substance P are likely to be mediated by their specific receptors found also in pituitary gland membranes (18, 28, 45).

A striking feature that arises from the comparison of the effect of neuropeptides upon LH secretion in the same assay system is the diversity of responses exhibited by the gonadotrophs. The mechanisms underlying the effect of neuropeptides on LH release have not been fully clarified, but it is conceivable that they may alter diverse links in the chain of events that start with GnRH binding to its receptor and end with the extrusion of the secretory granules. For example, opioid agonist or antagonist produce changes in the number of GnRH receptors (2), phorbol esters up-regulate GnRH receptors (17) and NPY produces an allosteric in-

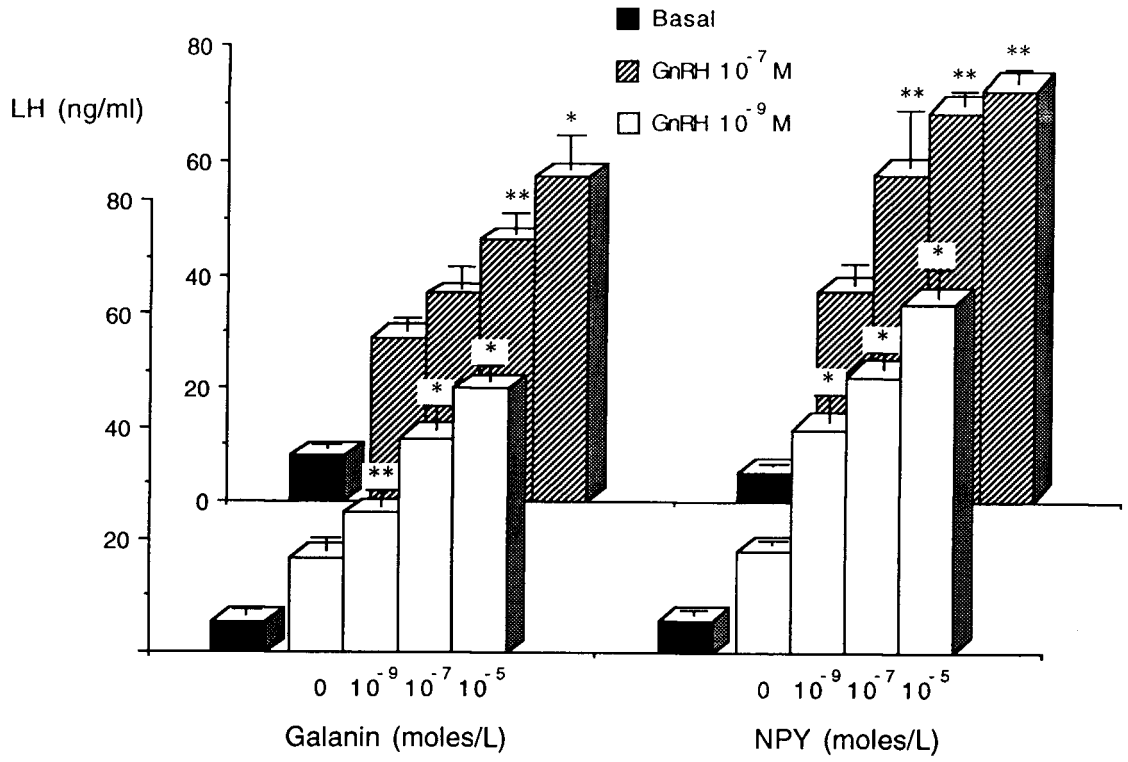


Fig 6. *Effects of galanin and neuropeptide Y (NPY) on GnRH-stimulated LH release.* After 3 days in culture, rat pituitary cells ($1.5 \times 10^5$ cells/well) were preincubated in the presence of increasing doses of galanin and NPY, for 30 min, and then were coincubated with GnRH $10^{-9}$ M or $10^{-7}$ M, for 2 h at 37° C. Media were collected at the end of the experiment and their LH contents determined by ELISA. Bars represent the means ± SD's of 8 replicates. * $p < 0.001$, ** $p < 0.005$ and *** $p < 0.05$ *vs* GnRH alone. Black bars represent basal LH secretion.

crease in GnRH binding to its receptor (33). The activation of intracellular messenger systems, such as adenylate cyclase and cAMP-dependent (39) or calcium/phospholipid-dependent protein kinases (40) also augment LH release in response to GnRH or other secretagogues under conditions in which basal hormone release is little affected. The diversity of responses to different neuropeptides under identical conditions, as reported here, offers great potential as a model to investigate the intracellular mechanisms involved in the control of LH release.

The present report adds support to the physiological relevance of neuropeptides in the regulation of LH secretion because the concentrations that were effective are comparable to intrapituitary and/or pituitary portal blood concentrations reported for galanin (24, 32), neurotensin (10), NPY (25, 31), substance P (28) and ß-endorphin (12, 44). Previous evidence that supports a physiological role of these neuropeptides is that the administration of antisera against ß-endorphin (11), or NPY (13), or substance P (9) or neurotensin (42) modified plasma LH levels. The demonstration of direct effects of several neuropeptides at physiological concentrations supports the concept that gonadotrophs are regulated by a more diversified control system than previously recognized. The most interesting example of pituitary control by multiple factors is the case of corticotrophs, in which the primary regulatory action of corticotrophin-releasing hormone (35) is potentiated and/or supplemented by the stimulatory effects of oxytocin (14) and vasopressin (15) and by catecholamines and angiotensin II (15).

Some of our results are not in full agreement with previous reports (5, 7, 20, 24, 26, 36, 43, 45) and this may be explained by methodological differences. For example, dispersed pituitary cells maintained in culture increase their rate of hormone secretion

5- to 20-fold respect to hemipituitaries (41). The method of cell dispersion, composition of the culture medium and cell density have distinct effects on cell functioning (4), and responsiveness to GnRH changes according to the age and sex of immature rats (34, 37, 38). The importance of these variables makes essential to compare the effects of neuropeptides under identical experimental conditions.

In summary, under the conditions used in this study, opioid peptides are preferentially inhibitory, while galanin, substance P, NPY and neurotensin are preferentially stimulatory on LH secretion. Galanin may account for that portion of the LH-releasing activity of ME extracts not explained by GnRH. This is based on the present observations that galanine increased basal and GnRH-stimulated LH secretion, effects which were not blocked by Nal-Glu. This action of galanin is thus not mediated by the GnRH receptor. The ability of different neuropeptides to modulate LH release at physiological concentrations indicates that regulatory signals complementary to the GnRH system are available for the hypothalamic control of reproductive function. The gonadotrophs in turn offer a wide variety of responses to these neuropeptides and their combinations. Altogether, the data lend further support to a role of galanin in the control of LH secretion.

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REFERENCES

1. ARONIN N, COSLOVSKY R, LEEMAN S (1986) Substance P and neurotensin. Annu Rev Physiol 48: 537-549
2. BARKAN AL, REGIANI S, DUNCAN JA, PAPAVASILIOU S, MARSHALL JC (1983) Opioids modulate pituitary receptors for gonadotropin-releasing hormone. Endocrinology 112: 387-389
3. BATTMANN T, M'ELIK S, JEANJEAN B, KERDELHUE B (1991) In-vivo inhibition of the preovulatory LH surge by substance P and in-vitro modulation of gonadotropin-releasing hormone-induced LH release by substance P, oestradiol and progesterone. J Endocrinol 130: 169-175
4. BEN-JONATHAN N, PELEG E, HOEFER MT (1983) Optimization of culture conditions for short-term pituitary cell culture. In: COLOWICK SP, KAPLAN NO (eds) Methods in Enzymology. Vol 103. New York: Academic Press. pp 249-257
5. BLANK MS, FABBRI A, CATT KJ, DUFAU ML (1986) Inhibition of luteinizing hormone release by morphine and endogenous opiates in cultured pituitary cells. Endocrinology 118: 2097-2101
6. BLUMENFELD Z, KUHN RW, JAFFE RB (1986) Corticotropin-releasing factor can stimulate gonadotropin secretion by human fetal pituitaries in superfusion. Am J Obstet Gynecol 154:606-612
7. CAICEDO L, SANCHEZ F (1986) Direct action of opioid peptides and naloxone on gonadotropin secretion by cultured rat anterior pituitary cells. Life Sci 38: 617-625
8. CROWLEY WR, HASSID A, KALRA SP (1987) Neuropeptide Y enhances the release of luteinizing hormone (LH) induced by LH-releasing hormone. Endocrinology 120: 941-945
9. DEES WL, SKELLEY CW, KOZLOWSKI GP (1985) Central effects of an antagonist and an antiserum to substance P on serum gonadotropin and prolactin secretion. Life Sci 37: 1627-1631
10. ECKLAND DJA, LIGHTMAN SL (1985) Neurotensin is secreted into the rat hypothalamo-hypophyseal portal blood. Regul Pept 13: 98
11. FORMAN LJ, SONNTAG WE, MEITES J (1988) Elevation of plasma LH in response to systemic injection of ß-endorphin antiserum in adult male rats. Proc Soc Exp Biol Med 173: 14-16
12. GENTLEMAN S, ROSS M, LOWNEY LI, COX BM, GOLDSTEIN A (1976) Pituitary endorphins. In: KOSTERLITZ HW (ed) Opiates and endogenous opioid peptides. Amsterdam: North Holland. pp 27-34
13. GHOSH P, DEBELYUK L (1990) Effect of antiserum to neuropeptide Y on LH secretion in normal and castrated transgenic mice with bovine growth hormone gene expresion. Endocrine Society, 72nd Annual Meeting, Atlanta, GA, USA. Abstr 1253
14. GIBBS DM, VALE W, RIVIER J, YEN SSC (1984) Oxytocin potentiates the ACTH-releasing activity of CRF (41) but not vasopressin. Life Sci 34: 2245-2249
15. GILLIES G, LINTON EA, LOWRY PJ (1982) Corticotrophin-releasing activity of the new CRF is potentiated several times by vasopressin. Nature 299:355-357
16. HART GR, GOWING H, BURRIN JM (1992). Effects of a novel hypothalamic peptide, pituitary adenylate cyclase-activating polypeptide, on pituitary hormone release in rats. J Endocrinol 134: 33-41
17. HUCKLE WR, Mc ARDLE CA, CONN PM (1988) Differential sensitivity of agonist- and antagonist-occupied gonadotropin-releasing hormone receptor to protein kinase C activators. J Biol Chem 263: 3296-3302
18. KALRA SP, CROWLEY WR (1992) Neuropeptide Y: A novel neuroendocrine peptide in the control of pituitary hormone secretion and its relation to luteinizing hormone. Front Neuroendocrinol 13: 1-46
19. KALRA SP, KALRA PS (1983) Neural regulation of luteinizing hormone secretion in the rat. Endocrine Rev 4: 311-351

20. KERDELHUE B, KHAR A, DENAY D, LANGLOIS Y, BERNNARDO T, LIWSKA J, JUTISZ M (1979) Inhibition *in vitro* par la substance P de l'excretion des gonadotropines induites par le LHRH a partir de cellules antehypophysaires de rat in culture. C R Acad Sci [D] Paris. 287: 879-882
21. LEIVA LA, DE LA LASTRA M (1992) Enzyme-linked immunosorbent assay for rat and human luteinizing hormone. Neuroendocrinology 56: 660-665
22. LEIVA LA, DE LA LASTRA M (1993) LH-releasing activity of hypothalamic extracts not attributable to GnRH. Neuroendocrinology 57: 615-620
23. LIGHTMAN SL, NINKOVIC M, HUNT SP, IVERSEN LL (1983) Evidence for opiate receptors on pituicytes. Nature 305: 235-236
24. LOPEZ FJ, MERCHENTHALER IM, CHING M, WISNIEWSKI MG, NEGRO-VILAR A (1991) Galanin: A hypothalamic-hypophysiotrophic hormone modulating reproductive functions. Proc Natl Acad Sci USA 88: 4508-4512
25. McDONALD JK, KOENIG JI, GIBBS DM, CALLINS P, NOE BD (1987) High concentrations of neuropeptide Y in pituitary portal blood of rats. Neuroendocrinology 46: 538-541
26. McDONALD JK, LUMPKIN MD, SAMSON WK, McCANN SN (1985) Neuropeptide Y affects secretion of luteinizing hormone and growth hormone in ovariectomized rats. Proc Natl Acad Sci USA 82: 561-564
27. MERCHENTHALER I, LOPEZ FJ, NEGRO-VILAR A (1991) Colocalization of galanin and luteinizing hormone-releasing hormone in a subset of preoptic hypothalamic neurons: Anatomical and functional correlates. Proc Natl Acad Sci USA 87: 6326-6330
28. MIKKELSEN J, LARSEN P, MOLLER M, VILHARDT H, SOERMARK T (1989) Substance P in the median eminence and pituitary of the rats: demonstration of immunoreactive fiber and specific binding sites. Neuroendocrinology 50: 100-108
29. MIYAKE A, YEN SSC (1981) Direct in vitro stimulation of pituitary LH release by alpha melanocyte stimulating hormone. Life Sci 29: 2637-2641
30. MORRIS BJ (1989) Neuronal localization of neuropeptide Y gene expression in rat brain. J Comp Neurol 290: 358-368
31. O'HALLARAN D, JONES P, GHATEI M, DOMIN J, BLOOM S (1990) The regulation of neuropeptide expression in rat anterior pituitary following chronic manipulation of estrogen status: a comparison between substance P, neuropeptide Y, neurotensin and vasoactive intestinal peptide. Endocrinology 127: 11463-11469
32. PALKOVITS M, RÖKAEUS A, ANTONI FA, KISS A (1987) Galanin in the hypothalamo-hypophyseal system. Neuroendocrinology 46: 417-423
33. PARKER SL, KALRA SP, CROWLEY WR (1991) Neuropeptide Y modulates the binding of a gonadotropin-releasing hormone (GnRH) analogue to anterior pituitary GnRH receptor sites. Endocrinology 128: 2309-2316
34. RAMALEY JA (1982) Pituitary gonadotropin-releasing hormone responsiveness in the prepubertal period: Effect of delayed puberty onset. Neuroendocrinology 34: 387-394
35. RIVIER C, PLOTSKY PM (1986) Mediation of corticotropin-releasing factor (CRF) of adenohypophyseal hormone secretion. Annu Rev Physiol 48:475-494
36. SHANGOLD GA, MILLER RJ (1990) Direct neuropeptide Y-induced modulation of gonadotrope intracellular calcium transients and gonadotropin secretion. Endocrinology 126: 2336-2342
37. SPONA J, LUGER O (1973) In vitro responsiveness of male rat pituitaries of different ages to LH-releasing hormone (LHRH). FEBS Lett 32: 49-51
38. SPONA J, LUGER O (1973) In vitro stimulation of LH release by LH-RH in female rat pituitaries of different ages. FEBS Lett 32: 52-54
39. TURGEON JL, WARING DW (1986) cAMP augmentation of secretagogue-induced luteinizing hormone secretion. Am J Physiol 250: E62-E68
40. TURGEON JL, WARING DW (1986) Modification of luteinizing hormone secretion by activators of $Ca^{2+}$/phospholipid-dependent kinase. Endocrinology 118: 2053-2058
41. VALE W, GRANT G, AMOSS M, BLACKWELL R, GUILLEMIN R (1972) Culture of enzymatically dispersed anterior pituitary cells: Functional validation of a method. Endocrinology 91: 562-572
42. VIJAYAN E, CARRAWAY R, LEEMAN S, McCANN SM (1987) Use of antisera to neurotensin to reveal a physiological role for the peptide in control of prolactin and LH release in the rat. Endocrinology (Suppl) 120: 86
43. VIJAYAN E, McCANN SM (1979) In vivo and in vitro effects of substance P and neurotensin on gonadotropin and prolactin release. Endocrinology 105: 64-68
44. WEHRENBERG WB, WERDLAW SL, FRANTZ AG, FERIN M (1982) ß-endorphin in hypophyseal portal blood: variations throughout the menstrual cycle. Endocrinology 111: 879-881
45. WORMALD PJ, MILLAR RP, KERDELHUE B (1989) Substance P receptors in human pituitary: A potential inhibitor of luteinizing hormone secretion. J Clin Endocrinol Metab 69: 612-615
46. ZAR JH (1974) Multiple Comparisons. In: McELROY WD, SWANSON CP (eds) Biostatistical Analysis. Englewood Cliffs, NJ: Prentice-Hall Inc. pp 151-162.