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Stimulation of Luteinizing Hormone-Releasing Hormone (LHRH) Gene Expression in $GT_{1–7}$ Cells by Its Metabolite, LHRH-(1–5)

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Given the central role of the decapeptide LHRH in reproduction and reproductive behavior, it is important to focus on delineating the possible effects of this gene and its products in the regulation of hormone-dependent reproductive processes. In the female, ovulation is preceded by a marked increase in LHRH release; the increase in LHRH release culminates in a preovulatory LH surge, which coincides with a period of sexual receptivity. In contrast to the belief that the proteolytic metabolism of LHRH serves only as a degradative process that removes excess LHRH and attenuates signal transduction through the LHRH receptor, we hypothesized that a metabolite of the decapeptide, LHRH-(1–5), can directly regulate LHRH neuronal function. This study demonstrates the ability of LHRH-(1–5) peptide to regulate LHRH gene expression in the LHRH neuronal cell line, the $GT_{1–7}$ cell. The results show that LHRH-(1–5) stimulated LHRH gene expression at the posttranscriptional level. In contrast to the LHRH suppression of its own gene expression, the coadministration of LHRH with the metalloendopeptidase, EC 3.4.24.15, an endopeptidase known to cleave LHRH to form $LHRH_{1–5}$, shows a reversal of effect, a stimulation of LHRH gene expression. Finally, the effect of LHRH-(1–5) on LHRH gene expression appears to be mediated by the calcium/calmodulin-dependent protein kinase. The present study supports the hypothesis that the physiological metabolite of LHRH, LHRH-(1–5), is functionally capable of regulating the reproductive neuroendocrine system. (*Endocrinology* 146: 280–286, 2005)

A WIDE BODY of literature has revealed the importance of LHRH (also referred to as GnRH) in the control of reproduction. Those LHRH neurons that comprise the final common pathway for the control of gonadotropin secretion are widely scattered in the basal forebrain region in which their axons are directed to the median eminence. Regulation of the anterior pituitary is mediated by the release of LHRH into the hypophyseal portal vessels and its subsequent delivery to the target. The LHRH terminal field therefore represents the final interface between neural and vascular elements that transport secreted LHRH to the anterior pituitary to elicit release of gonadotropins. Once released from the neurovasculature, LHRH must diffuse to the basal lamina of the brain and traverse the extracellular matrix to reach the fenestrated capillaries for transport to the pituitary in which it exerts its final amplified action.

Not only is LHRH known to regulate pituitary function, but it is also known for its nonpituitary actions in the brain and peripheral tissue. Indirect evidence comes from the identification of LHRH receptors in many regions including the hippocampus and medial basal hypothalamus (1–3). In addition, frequent sampling of cerebral spinal fluid (CSF) shows that LHRH is secreted into the CSF of the ventricle in many vertebrate species (4–6). The temporal pattern of LHRH concentration in the third ventricle coincides with the pulsatile LH profile in the periphery. Moreover, other studies show that LHRH functions act directly on olfactory receptor neurons (7), sympathetic ganglia (2, 8), ovarian tissue (9), and LHRH neurons (1, 10–13).

It is well characterized that LHRH can autoregulate its own secretion or biosynthesis. For example, central or peripheral administration of LHRH can alter the secretion and tissue content of LHRH and serum LH release (10, 13, 14). Recent reports (11, 15) showed that LHRH also alters its gene expression in the rat, presumably through an autoshort-loop feedback mechanism. Whereas these studies suggest that LHRH may regulate its own cellular function and expression, a small number of studies has suggested that a physiologically produced metabolic product of LHRH, LHRH-(1–5), may be involved in regulating reproductive function (16–18). Here we tested our hypothesis in $GT_{1–7}$ cells, an immortalized mouse LHRH neuron, that the pentapeptide, LHRH-(1–5), directly regulates LHRH gene expression.

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Abbreviations: AIP, Autocamtide-2-related inhibitory peptide; AVP, vasopressin; CaM, calmodulin-dependent protein (kinase); CSF, cerebral spinal fluid; EP24.15, EC 3.4.24.15; Ki, inhibitory constant; NMDA, *N*-methyl-D-aspartate.

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Materials and Methods

Cell culture

$GT_{1–7}$ cells were grown in a 1:1 media comprised of DMEM and Ham's F12 (Life Technologies, Inc., Invitrogen Corp., Carlsbad, CA) supplemented with 10% fetal calf serum (Hyclone, Logan, UT) and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin; Sigma, St.

Louis, MO) and maintained at 37 C with 5% CO_2. When cells attain 80% confluency, they were subcultured in a 1:4 ratio (Nunc, Rochester, NY). Before the experiment, the GT_{1-7} cells were subcultured to 6-well, 3.5-cm dishes and grown to approximately 80% confluency. The medium was changed to serum-free and antibiotic-free medium 1 h before the treatment. The end of the 1-h serum deprivation served as t = 0 for all experiments described here.

Reagents

Unless otherwise stated, reagents were purchased from Sigma. EGTA was purchased from Sigma (catalog no. E4378). The following inhibitors were all purchased from Calbiochem (San Diego, CA): the L-type calcium channel blocker, calciseptine (Ki = 290 nM) (19) (catalog no. 208274); and the calmodulin-dependent protein (CaM) kinase inhibitors, KN62 [(1-[*N*,*O*-bis-(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine)] [inhibitory constant (Ki) = 0.1 μM] (20) (catalog no. 422706), KN93 [2-(*N*-[2-hydroxyethyl)]-*N*-(4-methoxybenzenesulfonyl)[amino-*N*-(4-chlorocinnamyl)-*N*-methlyamine)] (Ki = 370 nM) (catalog no. 422708) (21), and autocamtide-2-related inhibitory peptide (AIP; Ki = 40 nM) (22, 23) (catalog no. 189482). The negative control for KN93, KN92 (catalog no. 422709), was also purchased from Calbiochem. The peptides bradykinin-(1–5) was purchased from Sigma (catalog no. B-1401) and IGF-(1–3) from Bachem (catalog no. H2468). LHRH-(1–5) was either purchased from Bachem (catalog no. H4080) or synthesized (Michael Flora, Biomedical Instrumentation Center, Uniformed Services University of the Health Sciences) in addition to scrambled or base-substituted pentapeptides. Bradykinin-(1–5), IGF-(1–3), LHRH-(1–5) and their scrambled controls, AIP, and calciceptine were initially dissolved in water at 1 mM and subsequently serially diluted with culture media to its final concentration. The final water to media ratio was never greater than 1:1000. EGTA was diluted directly into culture media. KN62 was initially dissolved in dimethylsulfoxide and used at a final dimethylsulfoxide dilution of 0.01% or lower. The controls included the vehicle at the same concentration.

RNase protection assay

At the end of each treatment, RNA from the GT_{1-7} cells was extracted (24, 25). Total cytoplasmic and nuclear RNA from the cytoplasmic suspension and the nuclear pellet, respectively, were extracted as previously described (23, 24) and stored under ethanol at −20 C. Total nuclear RNA and 1 μg total cytoplasmic RNA were resuspended in 20 μl hybridization solution [0.1 M EDTA (pH 8) and 4 M guanidine thiocyanate (final pH 7.5)] for RNase protection assay (26–28). Two DNA subclones containing LHRH complementary sequences were used as probes: 1) a 443-bp LHRH cDNA sequence that includes the entire cDNA subcloned into a pBS(+) vector (Stratagene, La Jolla, CA) spanning the unique *Eco*O109I and *Xba*I restriction sites; and 2) a proLHRH genomic fragment covering 383 bp of the intron A-exon 2-intron B junction (A2B) and subcloned in the *Spe*I and *Hind*III sites of a pBS(+) vector. The A2B riboprobe measured the LHRH primary transcript RNA. We have previously shown that changes in nuclear primary transcript are an accurate, albeit indirect, representation of gene transcription, as confirmed by transcription run-on studies in the GT_{1-7} cell line (29).

Quantitative ribonuclease protection assay was performed as described previously (24). For the standard curves, probes were mixed with increasing known amounts of LHRH cDNA (0–100 pg) and A2B (0–50 pg) reference RNAs. Samples and standards were allowed to hybridize for 16–18 h at 30 C; the remainder of the assay was conducted as described previously (24). Gels were exposed to a PhosphorImager screen (Molecular Dynamics, Sunnyvale, CA) for 5 d of quantitation using a PhosphorImager. The ImageQuant software (version 1.11, Molecular Dynamics) was used to determine the amount of radioactivity in each sample; this amount of radioactivity is defined as the summation of the intensities of all pixels within a defined area for standard curves and samples. Subsequently the background, which is defined here as average of all the pixel values lining the perimeter of each defined area, is subtracted from each pixel in the total volume. Finally, the amount of each sample is calculated by comparing each of the background-corrected volumes determined by the software to reference standard LHRH RNA using regression analysis.

Statistics

All experiments were conducted in triplicate and each triplicate repeated at least three times. For statistical analysis, results of triplicate cultures for each experiment were considered to one independent variable; all experiments were repeated at least three times, for an n greater than or equal to 3 per treatment. All data were subjected to Hartley's test for homogeneity before statistical analyses; if deemed necessary, the data were transformed (square root of each data point). Differences in mean RNA concentration were determined by an ANOVA followed by a *post hoc* comparison using Fisher's least significant difference test (significance at $P < 0.05$). For time-course studies, a two-way ANOVA was conducted.

Results

LHRH-(1–5) increased LHRH gene expression

As illustrated in Fig. 1, LHRH-(1–5) treatment of GT_{1-7} cells increased LHRH gene expression (cytoplasmic mRNA) in a time- and dose-dependent manner. Cells treated with the pentapeptide for 1 h increased ($P < 0.05$) LHRH mRNA levels at 10, 100, and 1000 nM concentrations. Compared with their respective controls, LHRH-(1–5) also increased LHRH mRNA levels in an acute and transient manner, with the greatest effect on gene expression occurring at 1 and 2 h. The effect ($P < 0.05$) was diminished by 4 h but still remained significant.

Unlike the effect on cytoplasmic LHRH mRNA levels, 100 nM LHRH-(1–5) for 1 h did not elicit a change in nuclear

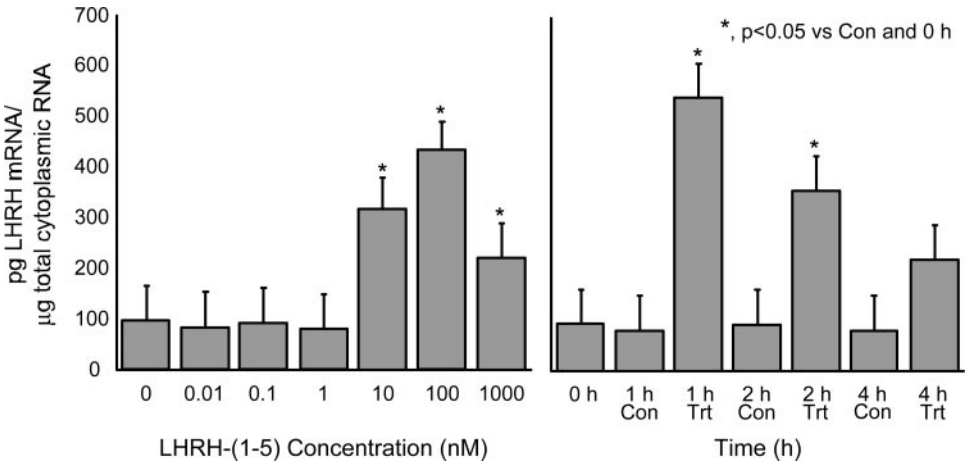

FIG. 1. The effect of LHRH-(1–5) dose (*left panel*) and time (*right panel*) treatment on LHRH gene expression in GT_{1-7} cells. Dose effects (*left panel*) were determined in GT_{1-7} cells using a range of LHRH-(1–5) concentrations (0, 0.1, 1, 10, 100, 1000 nM) for 1 h. To determine the time effect (*right panel*), GT_{1-7} cells were treated with 100 nM LHRH-(1–5) for 0 (less than 2 min exposure before setting on ice), 1, 2, and 4 h with LHRH-(1–5) and in parallel to vehicle control. Each *bar* represents the mean ± SE of the amount of cytoplasmic LHRH mRNA (picograms LHRH mRNA per milligram total cytoplasmic RNA) (n = 4 per treatment group). con, Vehicle control; Trt, treatment.

LHRH primary transcript levels (Fig. 2). Treatment of GT_{1-7} cells with LHRH-(1–5) also did not affect the LHRH primary transcript levels at 2 and 4 h (data not shown).

LHRH-(1–5) increased LHRH gene expression specifically

Functional specificity was tested by comparing the effect of LHRH and LHRH-(1–5) on its ability to autoregulate its cytoplasmic mRNA levels. The experiments in this section were conducted with cells treated for 1 h. In the first experiment, GT_{1-7} cells treated with 100 nM LHRH suppressed ($P < 0.05$), whereas those treated with LHRH-(1–5) stimulated, LHRH gene expression (Fig. 3). In the second experiment, addition of 20 μg/ml EP24.15, the neuropeptidase producing the LHRH-(1–5) fragment, with 100 nM LHRH, reversed ($P < 0.05$) the LHRH inhibition of LHRH gene expression (Fig. 4). In our hands, LHRH released from GT_{1-7} cells in the media was several orders of magnitude lower (3–40 pM) than the peptide levels added (100 nM) under the conditions tested (our unpublished observation). In addition, the levels of EP24.15 released by GT_{1-7} cells under the experimental conditions were found to be extremely low (our unpublished data), amounts insufficient for the effective cleavage of LHRH in the media (30). The addition of 20 μg/ml EP24.15 did not affect basal LHRH mRNA levels (data not shown).

Another way to test for specificity was to determine the effect of other small peptides and analogs of LHRH-(1–5). In contrast to the effect of LHRH-(1–5), other small peptides such as IGF-(1–3) and Bradykinin-(1–5) did not affect LHRH gene expression ($P > 0.05$) at any concentration tested (Fig. 5). We also tested the effect of analogs of LHRH-(1–5) on LHRH gene expression in GT_{1-7} cells. Scrambled peptides containing the same amino acid content as LHRH-(1–5) were not effective in eliciting an effect on LHRH gene expression in GT_{1-7} cells.

LHRH-(1–5)-induced increase in LHRH gene expression is calcium and CaM kinase dependent

Because it is well established that calcium and calcium-dependent signaling mechanisms are involved in LHRH neuronal function, we examined their role in the LHRH-(1–5)-stimulation of LHRH mRNA levels. Treatment of GT_{1-7} cells with 100 nM LHRH-(1–5) in the presence of the calcium ion chelator, EGTA, blocked the LHRH-(1–5)-induced increase in LHRH mRNA levels, suggesting the involvement of an extracellular Ca^{2+} flux mediating calcium-dependent signaling pathways in the cell (Fig. 6).

To further delineate which Ca2+ signaling pathways were involved, three different inhibitors of CaM kinase were tested to determine its involvement in mediating the LHRH-(1–5)-induced increase in LHRH gene expression. GT_{1-7} cells were treated with various concentrations of KN62 (Fig. 7A), KN93 (not shown), and AIP (Fig. 7B) with and without the

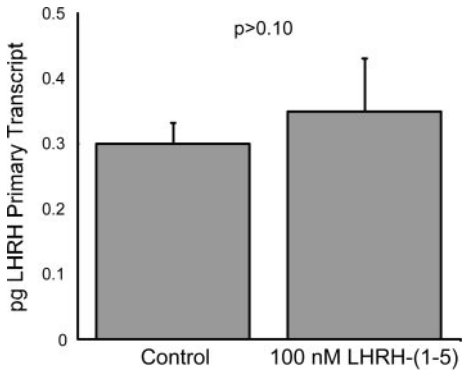

FIG. 2. The effect of LHRH-(1–5) treatment on LHRH primary transcript RNA concentration in GT_{1-7} cells. All cells were treated with vehicle or 100 nM LHRH-(1–5) for 1 h. Nuclear RNA was harvested from the cells at the end of the treatment period and expressed as the mean ± se of the amount of total nuclear primary transcript RNA (n = 4 per treatment group).

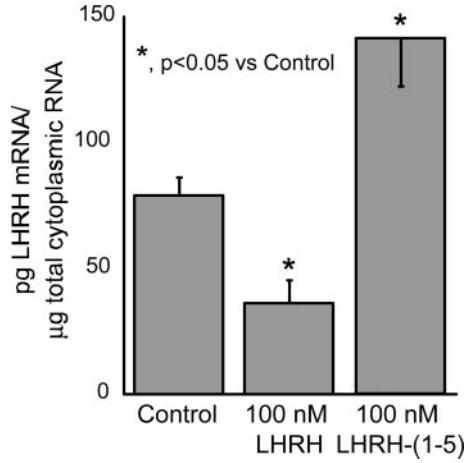

FIG. 3. The effect of 100 nM LHRH and 100 nM LHRH-(1–5) on cytoplasmic LHRH mRNA concentration in GT_{1-7} cells. All cells were treated with vehicle, 100 nM LHRH, or 100 nM LHRH-(1–5) for 1 h. Each *bar* represents the mean ± se of the amount of cytoplasmic LHRH mRNA (picograms LHRH mRNA per milligram total cytoplasmic RNA) (n = 3 per treatment group).

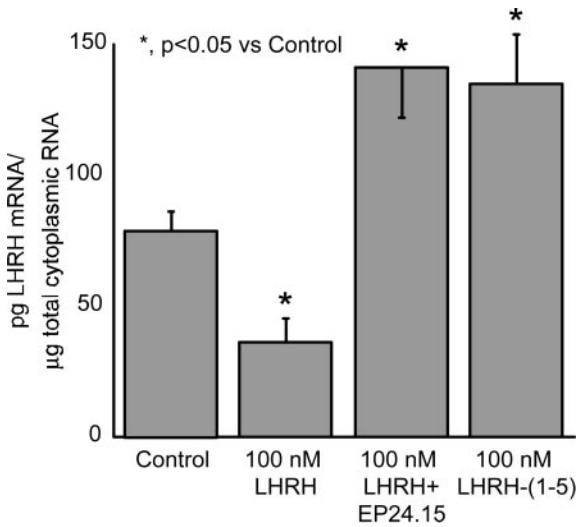

FIG. 4. Effect of the zinc metalloendopeptidase, EP24.15, and LHRH on LHRH gene expression in GT_{1-7} cells. Cells were serum starved for 1 h before treatment with vehicle, 100 nM LHRH, 100 nM LHRH with 20 μg/ml EP24.15 and 100 nM LHRH-(1–5) for 1 h. Each *bar* represents the mean ± se of the amount of cytoplasmic LHRH mRNA (picograms LHRH mRNA per milligram total cytoplasmic RNA) (n = 3 per treatment group).

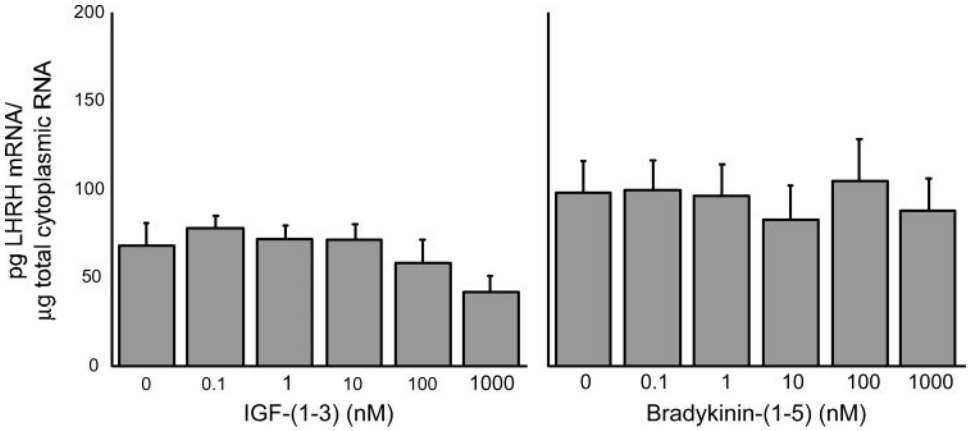

FIG. 5. Effect of IGF-(1–3) (*left panel*) and bradykinin-(1–5) (*right panel*) on LHRH gene expression in GT_{1-7}. Dose effects were determined in GT_{1-7} cells treated with a range of concentrations (0, 0.1, 1, 10, 100, 1000 nM) of both peptides for 1 h. Each *bar* represents the mean ± SE of the amount of cytoplasmic LHRH mRNA (picograms LHRH mRNA per milligram total cytoplasmic RNA) (n = 3 per treatment group).

presence of 100 nM LHRH-(1–5). Each inhibitor by themselves at the highest concentration did not affect LHRH gene expression but when administered with LHRH-(1–5) blocked LHRH-(1–5)-induced increase in LHRH gene expression. In these experiments with inhibitors, the concentration of each inhibitor used was based on its K_i and with the concentration that is 1 order of magnitude higher and 1 order of magnitude lower. Furthermore, the negative control of KN93, KN92, was also tested but not shown to have any effect on LHRH-(1–5)-induced increase in LHRH gene expression (data not shown).

Discussion

To our knowledge, this is the first report to show that the metabolic product of the decapeptide LHRH, LHRH-(1–5), may affect its own gene expression. In contrast to the negative autoregulatory feedback effect of LHRH on its gene expression, this metabolite stimulates LHRH mRNA levels in GT_{1-7} cells. In addition, the data also affirm previous studies suggesting the involvement of this pentapeptide in the regulation of LHRH and gonadotropin secretion (17, 18, 31).

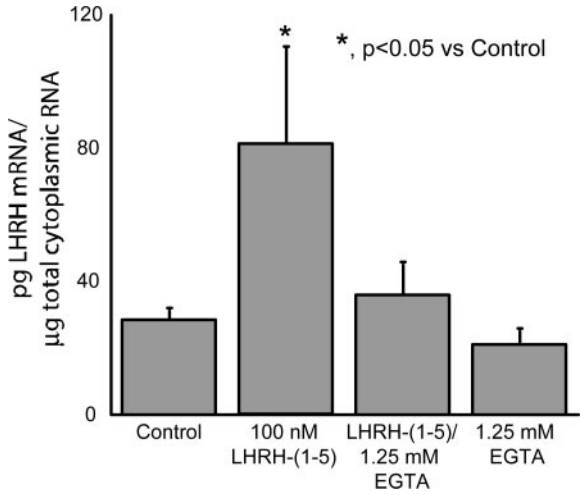

FIG. 6. Effect of chelating extracellular calcium on the LHRH-(1–5)-induced LHRH gene expression in GT_{1-7} cells. Cells were serum starved for 1 h before treatment with vehicle, 100 nM LHRH-(1–5), and/or 1.25 mM EGTA for 1 h. Each *bar* represents the mean ± SE of the amount of cytoplasmic LHRH mRNA (picograms LHRH mRNA per milligram total cytoplasmic RNA) (n = 4 per treatment group).

The ability of the LHRH neuronal system to autoregulate secretion and gene expression has been well established in *in vitro* and *in vivo* models. For example, it has been shown that LHRH treatment can inhibit its secretion in GT_1 cells (12). In addition, explant studies with the mediobasal hypothalamus also demonstrated LHRH inhibition of LHRH secretion (13, 17). Recent studies also indicate that LHRH inhibits LHRH gene expression in the rat (11, 15, 27). Whereas the mechanism and circuitry is not completely understood, it appears that the negative feedback effect is mediated by LHRH receptors because LHRH antagonists block these LHRH effects (11, 26, 27). Autoregulation of secretion and biosynthesis is not unique to the LHRH system but is common to neuropeptide systems in general. Administration of neuropeptides, such as oxytocin, somatostatin, and GHRH, either intraventricularly or in tissue culture has elicited strong effects on release of the respective hormones (13, 28).

Here we extend those studies with LHRH to its metabolite, LHRH-(1–5), which, unlike its parent decapeptide, has a stimulatory effect on LHRH gene expression in GT_{1-7} cells. The results from this study suggest that LHRH can have both stimulatory and inhibitory effects via a direct inhibitory effect by LHRH and a stimulatory effect by its degradation product, LHRH-(1–5). It is interesting to note that peptide concentrations of LHRH [and probably LHRH-(1–5)] can reach nanomolar concentrations in the CSF during the preovulatory LHRH/LH surge (4–6). The balance between LHRH and its metabolite on negative/positive ultrashort-loop feedback pathways suggests a plausible role in the recruitment of the preovulatory surge as well as the regulation of pulsatile LHRH release.

Whereas neuropeptides are important in transducing changes through their interactions with receptors in the extracellular milieu, they lack an active uptake mechanism for their clearance but rely on their eventual degradation by peptidases. A growing body of literature suggests that peptide degradation products, such as glucagon-like peptide-1 and vasopressin (AVP), are sufficiently stable to exert biological action (32–34). For example, AVP undergoes a stepwise aminopeptidase conversion process leading to the accumulation of several metabolites. The AVP metabolite [AVP-(4–9)], unlike its parent molecule, has little or no antidiuretic effects but has been shown to facilitate avoidance behavior and social learning and memory (35). A number of

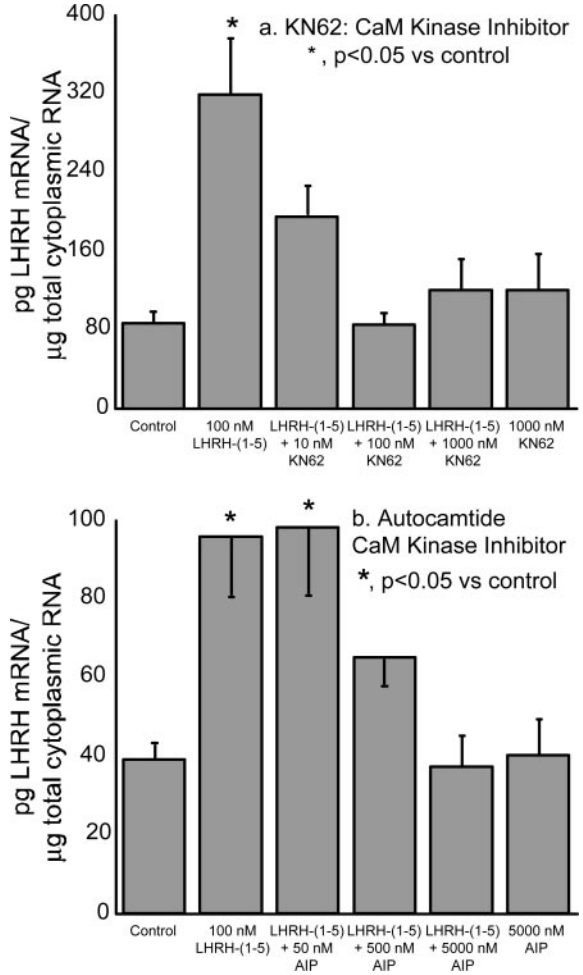

FIG. 7. Effect of the CaM kinase inhibitors, KN62 (A) and AIP (B), on the LHRH-(1–5)-induced LHRH gene expression in GT_{1-7} cells. All doses used represent 0.1, 1, and 10 × Ki for the respective inhibitors (10, 100, and 1000 nM for KN62; 40, 400, and 4000 nM for KN93; 50, 500, and 5000 nM for AIP). Each *bar* represents the mean ± SE of the amount of cytoplasmic LHRH mRNA (picograms LHRH mRNA per milligram total cytoplasmic RNA) (n = 3 per treatment group).

studies have shown that LHRH metabolites, including LHRH-(1–5), are present endogenously in the adult brain (36) and embryonic brain (37).

The best-characterized peptidase involved in LHRH metabolism is the zinc metalloendopeptidase EC 3.4.24.15 (EP24.15). EP24.15 is implicated in the metabolism of LHRH by cleaving the peptide bond linking the fifth and sixth amino acid to produce LHRH-(1–5). Localization of EP24.15 and its enzymatic activity support the involvement of EP24.15 in the modulation of hypothalamic LHRH neuronal function (31, 38). EP24.15 has been shown to be immunolocalized in the median eminence and preoptic area in close proximity with LHRH cell bodies and processes and can be colocalized with LHRH neurons (31). It is interesting to note that during embryonic development, EP24.15 has been localized in the same neuroanatomical regions as LHRH and LHRH-(1–5) (37). For example, an inverse relationship has been observed between LHRH content and its degradation activity within the median eminence during the preovulatory LH surge at a time when LHRH content was purportedly greatest (16, 37, 39, 40). In the rat, EP24.15 immunoreactivity is located within the perivascular space between LHRH axons and the portal vessels of the median eminence, whereas a systemically administered EP24.15 inhibitor enhanced the magnitude of the LH surge (31). Interestingly, EP24.15 immunoreactivity fluctuates on proestrous day of the rat estrous cycle within the median eminence with a peak expression coinciding with the ascending phase of the LH surge and a subsequent trough in expression post surge (41).

Presence of EGTA, a calcium ion chelator, blocked the LHRH-(1–5)-induced increase in LHRH mRNA levels in the GT_{1-7} cells. The data suggest the involvement of extracellular calcium in mediating the LHRH-(1–5)-induced increase in LHRH gene expression. This conclusion is further supported by the blockage of the LHRH-(1–5)-induced increase in LHRH gene expression by inhibitors of CaM kinase. This is consistent with other studies showing its involvement in regulating LHRH secretion in both the GT1 cell line and explant cultures of the median eminence from various animal species (41–43). Although the inhibitors are believed to be CaM kinase II specific, this is not stringently so. It has been shown that the two isoquinolonesulfonamides, KN-62 and KN-93, may also inhibit CaM kinases I and IV (44–46). However, the effect of AIP, which is not known to have an effect on any other CaM kinases than CaM kinase II, on LHRH gene expression in the present study suggest that CaM kinase II may be the target. In addition to the CaM kinase pathways, the signaling pathways involved are likely to be convoluted and complex with a multitude of kinases that cross-talk or overlap.

Studies are underway to define the role of CaM kinase II and other signaling pathways under the conditions of this study. It is interesting to note that Bourguignon *et al.* (17) suggest that LHRH-(1–5) may function as an antagonist to the *N*-methyl-D-aspartate (NMDA) receptor and thereby regulate LHRH release from retrochiasmatic explants. The link between L-type calcium channels and CaM kinase II with NMDA receptor function is well characterized (47, 48). Although LHRH perikarya and their axonal terminals express glutamate receptors (both NMDA and non-NMDA type) in the rodent (49–51), the effects may be species dependent. For example, NMDA has been shown to increase LHRH mRNA levels in rats but decrease LHRH mRNA levels in mice (52, 53). Nevertheless, it has been recently proposed that almost all LHRH neurons (99.5%) and axonal processes synthesize the vesicular glutamate transporter-2 and thus themselves may also produce glutamate (54, 55). This raises the possibility of an autocrine mechanism involving glutamate and a NMDA receptor antagonist that may be produced from the same neuron.

It is well recognized that LHRH gene expression is regulated transcriptionally and posttranscriptionally (24, 25, 29, 49, 56–58). The regulation of gene expression is the culmination of several steps: transcription to produce the primary transcript RNA, nuclear processing of this transcript into the mature mRNA through splicing, transport of the mRNA to the cytoplasm, and the final degradation of the mRNA in the cytoplasm. Because the time course for the LHRH-(1–5) effect is fast (initiated at 1 h or less), it is possible that the regulation

of LHRH gene expression by LHRH-(1–5) may be mediated transcriptionally and posttranscriptionally. Previous studies have shown, based on nuclear run-on experiments, that the measure of the primary RNA transcript serves as a good indicator of transcriptional rates (24–26, 29). Here treatment of GT_{1-7} cells with LHRH-(1–5) increased cytoplasmic LHRH mRNA levels (Fig. 1) but not the nuclear primary transcript levels (Fig. 2). That there is no concomitant change in the nuclear primary transcript LHRH level with the cytoplasmic LHRH mRNA levels suggests that the regulation of LHRH gene expression by LHRH-(1–5) occurs at least in part through a posttranscriptional level.

What is usually considered an intermediate degradation product of LHRH can exhibit a biological activity that is divergent from its precursor peptide. This dual ability of LHRH and its metabolic product to regulate LHRH gene expression increases the flexibility of such regulation for a physiological system that is sensitive to social and environmental regulation. This has implications in agonist design as well as a broader understanding of the neuroendocrine regulation of reproductive function. Future studies will determine the metabolism of this peptide, the intracellular signaling pathway, and the receptor mediating the action of LHRH-(1–5). It will be interesting to identify the role of LHRH-(1–5) in the autoregulation of LHRH neuronal function or expand it to encompass broader and multiple functions in the brain.

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