A preprogalanin cDNA from the turtle pituitary and regulation of its gene expression

John Yuh-Lin Yu,1* Chin-Hon Pon,2* Hui-Chen Ku,2 Chih-Ting Wang,2 and Yung-Hsi Kao2

1Institute of Cellular and Organismic Biology, Academia Sinica, Nankang, Taipei, Taiwan; and
2Department of Life Science, National Central University, Chung-li, Taiwan

Submitted 1 July 2006; accepted in final form 17 November 2006

Yu JYL, Pon C-H, Ku HC, Wang CT, Kao YH. A preprogalanin cDNA from the turtle pituitary and regulation of its gene expression. Am J Physiol Regul Integr Comp Physiol 292: R1649–R1656, 2007. First published December 7, 2006; doi:10.1152/ajpregu.00452.2006.—Galanin is a hormone 29 or 30 amino acids (aa) long that is widely distributed within the body and exerts numerous biological effects in vertebrates. To fully understand its physiological roles in reptiles, we analyzed preprogalanin cDNA structure and expression in the turtle pituitary. Using the Chinese soft-shell turtle (Pelodiscus sinensis order Testudines), we obtained a 672-base pair (bp) cDNA containing a 99-bp 5’-untranslated region, a 324-bp preprogalanin coding region, and a 249-bp 3’-untranslated region. The open-reading frame encoded a 108-aa preprogalanin protein with a putative 23-aa signal sequence at the NH2 terminus. Based on the location of putative Lys-Arg dibasic cleavage sites and an amidation signal of Gly-Lys-Arg, we propose that turtle preprogalanin is processed to yield a 29-aa galanin peptide with Gly1 and Thr29 substitutions and a COOH-terminal amidation. Sequence comparison revealed that turtle preprogalanin and galanin-29 had 48 – 81% and 76 – 96% aa identities with those of other vertebrates, respectively, suggesting their conservative nature. Expression of the turtle galanin gene was detected in the pituitary, brain, hypothalamus, stomach, liver, pancreas, testes, ovaries, and intestines, but not in the adipose or muscle tissues, suggesting tissue-dependent differences. An in vitro study that used pituitary tissue culture indicated that treatment with 17β-estradiol, testosterone, or gonadotropin-releasing hormone resulted in increased galanin mRNA expression with dose- or time-dependent differences, whereas leptin and neuropeptide Y reduced galanin mRNA levels. These results suggest a hormone-dependent effect on hypophysal galanin mRNA expression.

GALANIN, a 29-amino acid (aa) hormone, was first isolated from porcine intestine and used to stimulate contractions of rat smooth muscle and to cause hyperglycemia in dogs (52). Since its discovery, galanin has also been found to exhibit numerous actions in vertebrates. For example, it induces feeding and the release of pituitary hormones [i.e., luteinizing hormone (LH) and growth hormone (GH)], decreases circulating levels of oxytocin, plays an antinociceptive role in the spinal cord, is involved in gastrointestinal motility and autonomic function, inhibits memory and learning, and promotes smooth muscle contractions (12). Moreover, clinical investigations have shown that galanin levels and galanin receptors are related to human disorders, such as Alzheimer disease and obesity (12). In reptiles, galanin can control gastrointestinal blood flow (24) and female oviposition (29). Although its mechanism of action in these reptilian physiological processes remains unknown, galanin in mammals reportedly operates via Gs/Gq-protein-coupled receptors involving effector systems such as K+ channels, Ca2+ channels, and adenylate cyclase (3).

In mice and humans, the galanin gene contains six exons and five introns (14, 26), and its mRNA consists of 398–989 base pairs (bp), depending on the species (5, 8, 13, 14, 25–27, 35, 41, 42, 50). The open-reading frame encodes a mouse (124 aa) (26) and human (125 aa) (5, 14) preprogalanin protein. In vertebrates, mature galanin derived from the preprogalanin COOH terminus contains 29 aa and amides with glycine at the final residue (threonine or alanine) (2, 5, 8, 13, 14, 18, 25–27, 35, 41, 42, 50, 52, 56–59). However, macaque and human galanins have 30 aa and lack COOH-terminal amidation (5, 13), and there is also a second form of human galanin consisting of the first 19 NH2-terminal aa residues (5). These observations suggest that there are species-specific isoforms and lengths of galanin.

Despite the importance of galanin, little is known about preprogalanin cDNA structure in reptiles. Although the 29-aa reptilian galanin was first isolated from alligator stomach (56) and later from tortoise intestine (59), 5 of its 14 COOH-terminal aa in positions 16, 22, 23, 24, and 28 can vary. Despite these implied species-specific differences in the galanin nucleotide sequence, preprogalanin cDNA structure and expression have not been analyzed in these two reptilian taxa. Of the four reptilian orders (Testudines, Squamata, Crocodylia, and Rhynchocephalia), turtles and tortoises belong to Testudines and occupy an important position in the reptilian phylogeny. Thus deducing the aa sequences of preprogalanin and mature galanin from turtle galanin cDNA can allow comparison with those of alligators (56), tortoises (59), and other vertebrates (2, 5, 8, 13, 14, 18, 25–27, 35, 41, 42, 50, 52, 57, 58), helping to clarify species-specific differences. In addition, isolation of turtle preprogalanin cDNA provides a basis for exploring its regulation and tissue-specific expression in turtles.

In mammals, the widespread distribution of galanin in the central and peripheral nervous systems, the gastrointestinal tract, and reproductive system has been extensively described (12). With antiseraum raised against porcine galanin, galanin immunoreactivity was identified in the reptilian telencephalon, diencephalon, rhombencephalon, and oviduct, and its activity in nonreproductive lizard oviduct increased after 17β-estradiol (E2) administration (23, 29). Without mRNA expression data in turtles (23) and lizards (29), these immunohistological

* J. Y.-L. Yu and C.-H. Pon contributed equally to this work.

Address for reprint requests and other correspondence: Y.-H. Kao, Dept. of Life Science, College of Science, National Central Univ., Chung-li City, Taoyuan 32054, Taiwan (e-mail: ykao@cc.ncu.edu.tw).

http://www.ajpregu.org

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
results could not distinguish specific galanin-producing tissues in reptiles or demonstrate whether E2 directly or indirectly alters galanin mRNA levels. Estradiol, testosterone (T), progesterone, and gonadotropin-releasing hormone (GnRH) activate galanin gene expression in mammals, whereas insulin, leptin, and neuropeptide Y (NPY) inhibit it (12, 45, 47). Whether any of these sex steroid hormones and hypothalamic peptides exert direct effects on galanin mRNA expression in reptiles is unknown. Thus further reptile-based, in vitro tissue cultures free of organismal influences and using precise hormone concentrations should be excellent systems for studying the direct effects of these sex steroids and peptide hormones on galanin gene expression.

In this study, we used the Chinese soft-shell turtle to analyze the preprogalanin cDNA sequence in turtle pituitary; this species is commercially cultured in Taiwan and is easily accessible and economically important. In addition, we investigated whether galanin mRNA expression is tissue specific in this species and whether in vitro treatments with E2, T, GnRH, NPY, or leptin differentially affect hypothalamic galanin gene expression. We selected the pituitary as the target tissue because of reports that it expresses galanin in mammals (12) and because it is a major site of paracrine and endocrine interactions among galanin, other hormones, and brain peptides (45). E2, T, GnRH, NPY, and leptin were chosen because they can target the vertebrate pituitary and can regulate reproduction and energy homeostasis (16), as has been reported for galanin (6–7, 17, 28, 44–45). Our findings on the regulation of galanin mRNA levels suggest that this hormone plays a role in the control of the turtle hypothalamo-hypophyseal-gonadal axis.

MATERIALS AND METHODS

Chemical reagents. All materials (e.g., E2, GnRH, and BSA) were purchased from Sigma (St. Louis, MO) unless otherwise stated. NPY and leptin were obtained from Bachem (Torrance, CA). Because GnRH, NPY, and leptin were not homologously isolated from the Chinese soft-shell turtle and because turtle forms of these hormones were not commercially available, heterologous salmon GnRH, rat leptin, and NPY, or leptin differentially affect hypophyseal galanin gene expression. We selected the pituitary as the target tissue because of reports that it expresses galanin in mammals (12) and because it is a major site of paracrine and endocrine interactions among galanin, other hormones, and brain peptides (45). E2, T, GnRH, NPY, and leptin were chosen because they can target the vertebrate pituitary and can regulate reproduction and energy homeostasis (16), as has been reported for galanin (6–7, 17, 28, 44–45). Our findings on the regulation of galanin gene expression suggest that this hormone plays a role in the control of the turtle hypothalamo-hypophyseal-gonadal axis.

Animals. Chinese soft-shell turtles, Pelodiscus sinensis, were obtained from the Ching-Der commercial market near Nankang, Taipei, Taiwan. The turtles were ~1 year of age and weighed ~600 g. Animal experimental protocols were reviewed and approved by the Laboratory Animal Ethics Committee, Academia Sinica, Taipei, Taiwan.

RNA extraction, oligonucleotide primers, and RT-PCR. Total RNA was isolated from five turtle pituitaries using the RNA miniprep system kit (Viogene, Sunnyvale, CA). For the 3’-RACE system, cDNA was synthesized from 1 μM of 5’-RACE CDS primer [5’-T(N)2N3, N= A, C, G, or T and N1 = A, G, or C] and 1 μM of SMART IIA oligonucleotide (5’-AACAGTGTATCAACCGAGGTG-3’) using MLV-RT (Invitrogen) according to the SMART 5’-RACE kit. PCR was performed under conditions similar to those of the 3’-RACE system, except the annealing temperature was set to 64°C and the forward and reverse primers were the universal primer mix A and 5’-AGTAGATTGTTCAAGTGTCTCCACC-3’, respectively. The predicted product was nested by another PCR with the use of the forward and reverse primers 5’-AACAGTTGTTATCAACCGAGGTG-3’ and 5’-AGATGCAAATGCTCATAAAAATTTA-3’, respectively. Overlapping sequences of the nested PCR product and the previous 3’-RACE PCR product were used to identify a turtle preprogalanin cDNA.

For experiments studying the tissue-specific distribution of turtle galanin gene expression and its regulation, the reverse transcription and PCR reactions were similar to the 3’-RACE systems, except that the annealing temperature was set to 60°C. The forward and reverse primers were 5’-CTGCTGCAAAAGAAAAAGAGGTTG-3’ and 5’-AGTTGATTTGTTCAAGTGTCTCCACC-3’ for galanin and 5’-CCATGACCGTGCCATCCAGG-3’ and 5’-ACTACCTTGAAGATGCT-3’ (accession no. AY373753) for actin, respectively. The galanin and actin PCR products, respectively predicted to be 250 and 200 bp, were sequenced by Dr. Chip Biotechnology. An almost linear limit in the number of PCR amplifications for galanin was observed between 20 and 40 cycles compared with the β-actin standard. Thus 30 cycles of PCR amplification were subsequently used for all experiments. Agarose gel electrophoresis of the galanin and actin PCR products were visualized by ethidium bromide staining and UV transillumination and then quantified by a molecular imager (Bio-Rad Laboratories, Hercules, CA). On the basis of the scanning values, galanin cDNA levels in each sample were divided by levels of β-actin to correct the variance of RNA amount in each sample that was loaded on the gels. After correction to β-actin mRNA, galanin levels were expressed as a percentage of the control.

In vitro pituitary tissue culture. Pituitary tissues were incubated according to the method described by Chien et al. (10). Briefly, pituitaries were removed from adult male turtles, immediately washed twice in 1× Hanks buffer (GIBCO BRL), and then placed in 35-mm culture dishes. After extraneous tissues were removed, pituitaries were sliced into six to eight pieces and placed in sterile phenol red-free DMEM containing 1% (vol/vol) antibiotic and antimycotic agents (GIBCO BRL) in a humidified atmosphere of 95% O2-5% CO2 at 25°C. Pituitaries were preincubated for 0.3% BSA-supplemented medium for 2 h to reach the basal levels of galanin mRNA and then were treated with or without E2 (0.1–1.00 nM), T (0.1 and 10 nM), GnRH (0.1–100 nM), NPY (1–100 nM), and leptin (10 and 1,000 nM)
for the indicated time periods. After treatment, total RNA was analyzed with galanin mRNA levels by semi-quantitative RT-PCR, as described above. We should note that the exact physiological levels of E₂, T, GnRH, NPY, and leptin have not been determined in the Chinese soft-shell turtles and neither have the primary aa sequences of GnRH, NPY, and leptin. Accordingly, some doses of E₂ (0.1–10 nM), T (0.1 and 10 nM), GnRH (0.1–10 nM), NPY (1 and 10 nM), and leptin (10 nM) used in our study are potentially close to physiological circulating levels reported in other reptiles (9, 24, 37, 55) and vertebrates (4, 34, 47). Other high doses of E₂ (1,000 nM), GnRH (100 nM), NPY (100 nM), and leptin (1,000 nM) used in these studies potentially correspond to pharmacological levels.

Statistical analysis. Data are expressed as means ± SE. One-way ANOVA followed by the Student-Newman-Keuls multiple-range test was used to examine differences among multiple groups. Differences were considered significant at P < 0.05. All statistics were performed using SigmaStat (Jandel Scientific, Palo Alto, CA), and data were log transformed.

RESULTS AND DISCUSSION

Characterization of preprogalanin cDNA. An overlapping sequence of the 3’-RACE PCR and 5’-RACE PCR fragments from the total RNA of the turtle pituitary was used to identify a 672-bp galanin cDNA with a single potential initiation site 100 bases downstream from the most 5’ end and a single putative polyadenylation signal (AATAAA) 14 bases from the most 3’ end (Fig. 1A). The nucleotide sequence contained a 99-bp 5’-untranslated region, a 324-bp preprogalanin coding region, and a 249-bp 3’-untranslated region. The open-reading frame encoded a preprogalanin protein of 108 aa with a putative signal sequence of 23 aa at nucleotide positions 1 through 67. Multiple protein sequence alignment was performed by the CLUSTAL W program and the PileUP program from the Wisconsin GCG program (1, 53, 54), and the Blosum62 aa substitution matrix was used to calculate the protein sequence homology (20).

After the sequence comparison, the turtle preprogalanin cDNA and its deduced aa exhibited homologies with other vertebrate species (2, 5, 8, 13, 14, 18, 25–27, 35, 41, 42, 50, 52, 56–59) in the ranges of 50–80% and 48–81%, respectively (Fig. 1B). The deduced protein also contained a number of putative recognition sites for posttranslational modification by converting enzymes, including a Lys-Arg dibasic site at both COOH- and NH₂-terminus and COOH terminus, potentially yielding a 29-aa galanin peptide. The COOH-terminal aa of turtle galanin contained Thr, Gly, Lys, and Arg at positions 29, 30, 31, and 32, respectively, indicating the possible COOH-terminal amidation of the turtle galanin at Thr. This notion is consistent with reports on birds (27) and rodents (25, 26) but inconsistent with those published on primates (5, 13, 14). It has been demonstrated that the NH₂-terminal portion of galanin, particularly the first 15 aa, has been conserved across vertebrate species and is important for receptor interaction and galanin bioactivity in various tissues. The COOH-terminal portion of galanin, however, is variable, has no known bioactivity, and is responsible for the species specificity in vertebrates (12).

Accordingly, we observed that the first 15 NH₂-terminal aa deduced from the turtle galanin sequence were identical to those of most vertebrate species (2, 5, 8, 12–14, 18, 25–27, 35, 41, 42, 50, 52, 56–59) and that up to the first 7 of the 14 COOH-terminal aa differed from those reported in other species (Fig. 1B). The comparison indicated that turtle galanin-29 peptide shared 76–96% aa identity with other vertebrates, including 86% and 83% with a tortoise (59) and the alligator (56), respectively, suggesting its conserved nature among these species.
limitations did not allow for analysis of galanin expression in specific regions of the brain, pituitary, and hypothalamus of the *P. sinensis*. A study (23) that used the brain of the turtle *Mauremys caspica* and antiserum against porcine galanin indicated that galanin-like immunoreactive perikarya were present in the telencephalon (i.e., amygdaloid complex), diencephalon (i.e., infundibulum and nuclei hypothalamicus ventromedialis and posterior), and rhombencephalon (i.e., nucleus tractus solitari) but were absent from the mesencephalon. In addition, galanin-like immunoreactive nerve fibers were present in all regions containing labeled perikarya and in all brain divisions (i.e., nucleus fasciculi diagonalis in the telencephalon, nucleus paraventricularis in the diencephalon, substantia nigra in the mesencephalon, and nuclei of the reticular formation in the rhombencephalon) (23). This study did not determine whether the tissue-specific distribution of turtle galanin can explain its biological effects on gastrointestinal blood flow (24), activity in the gut wall (24), and induction of oviposition (29). An interesting finding was that the female turtle pituitary displayed galanin mRNA levels threefold higher than those of males (Fig. 2), also indicating sex-dependent differences in hypothalamic galanin expression in turtles and/or the possible stimulatory effect of sex steroid hormones on hypothalamic galanin gene expression in male turtles.

**Regulation of hypothalamic galanin gene expression by sex steroid hormones.** To further demonstrate whether gonadal hormones regulate hypothalamic galanin mRNA expression, we tested pituitary tissues with E2 or T for the indicated periods (Fig. 3). E2 increased the steady-state levels of galanin mRNA in a dose-dependent and time-dependent manner (Fig. 3A). The concentration of E2 that increased galanin mRNA levels by 125–250% was ∼10 nM for a range of 6–8 h of treatment. At 0.1 and 10 nM doses for 6 h of treatment, T was found to increase galanin mRNA expression by 48–60% (Fig. 3B). Although the pituitary of female turtles expressed more galanin mRNA than that of male turtles (Fig. 2), we found no statistically significant difference between T and E2 in effects on galanin mRNA expression. Galanin mRNA levels in pituitary treated with 10 nM E2 were, however, ∼65% higher ($P = 0.06$) than in pituitary treated with 10 nM T; thus we cannot completely rule out a possible dominant effect of E2 on galanin in turtles, as reported in the rat pituitary (47). Because E2, T,
and progesterone regulate galanin gene expression in mammals, it would be of interest to determine whether these sex steroids interact to control in vivo galanin expression sex specifically in turtles.

**Differences among GnRH, NPY, and leptin in the regulation of galanin mRNA expression.** The pituitary is well known as the target tissue for the hypothalamic hormones and peptides (45). Thus, we also assessed GnRH, NPY, and leptin regulation of hypophyseal galanin mRNA expression (Fig. 4). We found that treatment with 1 nM GnRH for 4, 8, 16, and 24 h increased galanin mRNA expression by 218%, 220%, 50%, and 40%, respectively (data not shown), suggesting a time-dependent GnRH effect on galanin expression in pituitary tissue. At 0.1–100 nM for 6 h of treatment, GnRH resulted in significant increases in the galanin mRNA, from 50–1,500% (Fig. 4A; data with 0.1 nM of GnRH treatment not shown). In contrast, 10 and 100 nM of NPY (Fig. 4B) and 10 and 1,000 nM of leptin (Fig. 4C) significantly decreased galanin mRNA levels by about 63%, 95%, 59%, and 95%, respectively. These experiments indicate hypothalamic hormone- or neuropeptide-specific effects on hypophyseal galanin mRNA expression in the Chinese soft-shell turtle consistent with those observed previously in mammals (12, 45).

Together with the finding that galanin is located in the hypothalamus and the pituitary of the Chinese soft-shell turtles, GnRH-induced changes in hypophyseal galanin gene expression may indicate a possible role for galanin in sex behaviors and reproductive endocrinology via coordination of the turtle hypothalamo-hypophyseal axis. The following observations indirectly support this contention. First, galanin-immunoreactive brain regions of the turtle *M. caspica* were predominantly seen in the perikarya of the infundibulum, preoptic area, nucleus periventricularis anterior, and nuclei hypothalamicus ventromedialis and posterior (23). Second, microinjection of galanin into the preoperc area or the intracerebroventricular site stimulates sex behavior in male and female rats (6, 7, 17). Third, mammalian studies have shown that galanin stimulates releases of LH, LH-releasing hormone, GH, prolactin, GH-releasing hormone, and corticotropin-releasing hormone and inhibits releases of oxytocin, adrenocorticotropic, and follicle-stimulating hormone (3, 12, 16, 45). NPY and leptin affect hypothalamic synthesis and galanin release in mammals, thus playing a role in energy homeostasis regulation (4, 12, 43). In addition, galanin induces feeding in satiated rats via paraventricular, lateral, and ventromedial hypothalamic sites (28, 44) where abundant galanin immunoreactivity was identified in turtles (23). Firm conclusions as to whether both NPY and leptin regulate energy homeostasis in the Chinese soft-shell turtle by acting on pituitary galanin mRNA expression require further in vivo studies.

A given target tissue is usually responsive to a number of different hormones. Accordingly, we examined whether E2 and leptin antagonize one another’s effects on hypophyseal galanin gene expression in turtles. To test this possibility, we measured hypophyseal galanin mRNA levels in pituitary tissues pretreated with 10 nM E2 for 6 h and then incubated with 10 nM leptin for another 6 h (Fig. 5). Compared with control, E2 alone increased galanin mRNA steady-state levels by 70%; in the presence of leptin, it did not alter galanin mRNA levels. Compared with the leptin-treated group, E2 blocked leptin suppression of galanin mRNA levels. We also measured hypophyseal galanin mRNA levels in pituitary tissues pretreated with 10 nM leptin for 6 h and then incubated with 10 nM E2 for another 6 h (Fig. 5). Compared with control, leptin alone reduced galanin mRNA steady-state levels by 42%; in the presence of E2, leptin did not alter galanin mRNA expression. Compared with the E2-treated group, leptin prevented E2-stimulated galanin mRNA expression. Finally, concurrent treatment with both E2 and leptin for 12 h caused no significant change in galanin mRNA levels in turtle pituitary compared with control.

These observations suggest that E2 works differently from leptin in regulating galanin gene expression. One possible explanation for these distinct effects is that E2 and leptin may differentially affect transcription or posttranscriptional stability of turtle galanin mRNA. The presence of estrogen receptor (ER) binding sites has been demonstrated within the human galanin gene to regulate estrogen action in the pituitary (21)
that E2 induces galanin gene expression in mouse pituitary in this contention receives indirect support from observations on the relationship between E2 and galanin expression. The data, SE bars are too small to be seen. a–cGroups with different letters are significantly different. Groups with different letters are significantly different (P < 0.05) from each other. *Groups with different letters are significantly different (P < 0.05) from each other.

and that E2 can stimulate rat pituitary galanin gene expression through a transcriptional mechanism, although not via post-transcriptional mRNA stabilization (38). Another explanation is that a signal transducer different from the many ER isoforms may be responsible for the different effects of E2 and leptin on galanin gene expression. This contention receives indirect support from observations that E2 induces galanin gene expression in mouse pituitary in an ER-independent manner (48); that galanin mRNA levels may differ from that of homologous peptide on hypophyseal galanin mRNA expression in turtles. According to previous laboratory observations (9–11, 55), these heterologous hormones can stimulate pituitary hormone expression in lower vertebrates, including lizards and Chinese soft-shell turtles. In addition, rat NPY used in this study is identical to human NPY, tortoise NPY, and alligator NPY (30, 59). Moreover, the salmon GnRH-10 peptide is identical to the major form of lizard LH-releasing hormone and has 80–90% aa identity with those of the alligator, rat, human, chicken, and turtle (Trachemys scripta) (32, 39, 40, 49). Unfortunately, the primary aa sequence of reptilian leptin remains unknown. Although the physiological levels of leptin in rats and lizards (Podarcis sicula) are in the ranges of 0.06–5.6 (4) and 0.025–0.2 nM (37), as determined with a multispecies leptin radioimmunoassay kit, circulating leptin levels in the lizard Sceloporus undulatus were reported to be very high, in the range of 62–218 nM (51). Although expression of the galanin gene in turtle pituitary was differently regulated by low doses of salmon GnRH (0.1–10 nM), rat NPY (1–10 nM), and rat leptin (10 nM) in this study, the results do not exclude the possibility that the potency of heterologous hormones in altering turtle galanin mRNA levels may differ from that of homologous hormones. It would be worthwhile in a future study to clarify this possibility.

In conclusion, we have characterized a 672-bp reptilian preprogalanin cDNA from the turtle pituitary and determined that the deduced aa sequences of preprogalanin, progalanin, and galanin are conserved among vertebrates. This finding increases our understanding of the phylogenetic similarities and diversities of galanin molecules in vertebrates. Although the galanin gene was differentially expressed in turtle tissues, expression of the hypophyseal galanin gene appeared to be sex specific.

Fig. 5. Interactive effect of E2 and leptin on galanin mRNA expression. Pituitary tissues isolated from Chinese soft-shell turtles were pretreated with either E2 (10 nM) or leptin (10 nM) for 6 h and then stimulated with either leptin or E2 for another 6 h (the groups symbolized by the arrow). The other 3 treatment groups were as follows: 1) E2 alone for 12 h, 2) leptin alone for 12 h, and 3) simultaneous addition of both E2 and leptin for 12 h. Control experiments had no hormone treatment at 12 h. Bands (top) show representative RT-PCR (duplicate assays of each hormonal treatment). The forward and reverse primers for the PCR system were 5′-CTGTCTGCAAAAGAAAAAGAGGTGG-3′ and 5′-AGGTAGATTGTCAAATGTCCACC-3′ for galanin and 5′-CCATGTCATGGCCATCCAGG-3′ and 5′-ACTACCTCATGAAGATCCGT-3′ for actin, respectively. Agarose gel electrophoreses of the galanin and actin PCR products were visualized by ethidium bromide staining and UV transillumination, and their sizes were predicted to be 250 and 200 bp, respectively. After normalization to actin mRNA, galanin levels in the experimental groups shown in the histogram (bottom) were expressed as a percent of the control. Data are expressed as means ± SE from 3 samples. In some data, SE bars are too small to be seen. *Groups with different letters are significantly different (P < 0.05) from each other.
dependent. Localization of galanin in the central nervous system, gastrointestinal system, and reproductive organs of the Chinese soft-shell turtle and the differential regulation of physiological and others were pharmacological, dose-related responses were obtained for all hormones tested. Firm conclusions about whether any of these in vitro effects of sex steroid and hypothalamic hormones can be used to explain their in vivo effects on pituitary galanin mRNA levels will also require more studies.

ACKNOWLEDGMENTS

We thank Drs. Jung-Tsun Chien and San-Tai Shen for technical assistance.

GRANTS

This work was supported by grants from the Academia Sinica and the National Science Council, Taiwan to J. Y.-L. Yu and from the National Science Council, Taiwan, and the University System of Taiwan, Taiwan, to Y.-H. Kao.

REFERENCES

3. Bartfai T, Hokfelt T, Langel U. Galanin—a neuropeptide in the central nervous system, gastrointestinal system, and reproductive organs of the Chinese soft-shell turtle and the differential regulation of physiological and others were pharmacological, dose-related responses were obtained for all hormones tested. Firm conclusions about whether any of these in vitro effects of sex steroid and hypothalamic hormones can be used to explain their in vivo effects on pituitary galanin mRNA levels will also require more studies.


