Published in final edited form as:

Neurosci Lett. 2008 June 13; 438(1): 96–101. doi:10.1016/j.neulet.2008.04.042.

PRENATAL EXPRESSION OF CHOLECYSTOKININ (CCK) IN THE CENTRAL NERVOUS SYSTEM (CNS) OF MOUSE

Paolo Giacobini^{1,2} and Susan Wray¹

¹Cellular and Developmental Neurobiology Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA

²Laboratory of Neurobiology, Department of Human and Animal Biology, University of Turin, Italy

Abstract

Cholecystokinin (CCK) is a peptide found in both gut and brain. Although numerous studies address the role of brain CCK postnatally, relatively little is known about the ontogeny of CCK expression in the central nervous system (CNS). Recent work revealed that CCK modulates olfactory axon outgrowth and gonadotropin-releasing hormone-1 (GnRH-1) neuronal migration, suggesting that CCK may be an important factor during CNS development. To further characterize the developmental expression of CCK in the nervous system, *in situ* hybridization experiments were performed. CCK mRNA expression was widely distributed in the developing mouse brain. As early as E12.5, robust CCK expression is detected in the thalamus and spinal cord. By E17.5, cells in the cortex, hippocampus, thalamus and hypothalamus express CCK. In addition, CCK mRNA was also detected in the external zone of the median eminence where axons of the neuroendocrine hypophysiotropic systems terminate. Our study demonstrates that CCK mRNA is expressed prenatally in multiple areas of the CNS, many of which maintain CCK mRNA expression postnatally into adult life. In addition, we provide evidence that regions of the CNS known to integrate hormonal and sensory information associated with reproduction and the GnRH-1 system, expressed CCK already during prenatal development.

Keywords

CCK; *in situ* hybridization; development; GnRH-1; median eminence

Expression of the gut/brain molecule cholecystokinin (CCK) has been examined in postnatal animals and found to be primarily expressed in two cell types: endocrine cells of the intestine and neurons of the CNS [1]. Within the CNS, CCK has been implicated in a variety of functions including regulation of food intake, nociception, learning processes, anxiety/panic and various neurological conditions [4,5,8,9,25]. In addition, CCK has been shown to be involved in activation of female reproductive behaviors and release of anterior pituitary hormones [10, 28], and CCK levels in several brain nuclei (medial preoptic nucleus, bed nucleus of the stria terminalis, paraventricular nucleus and basolateral amygdala) appear to be regulated by circulating gonadal steroids [21]. With respect to the latter functions, a central action of CCK

To whom correspondence should be addressed: Dr. Susan Wray, Building 35, Rm. 3A-1012, Bethesda, MD 20892-3714, Phone: (301) 496-6646 Fax: (301) 496-8578, wrays@ninds.nih.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

on the GnRH-1 system has been recently reported with CCK acting directly on GnRH-1 neurons to attenuate their neuronal activity [7]. GnRH-1 neurons are fundamental components of the hypothalamic-pituitary-gonadal axis. Episodic release of GnRH-1 from axon terminals in the median eminence (ME) of postnatal animals is essential for gonadotropin secretion from anterior pituitary cells and thus reproductive function [15].

Although well studied postnatally, few studies have addressed the ontogeny of CCK expression during brain development. Prenatally, CCK was observed in subpopulations of neural tube and neural crest cells in mouse embryos, E8.5–E9.5 [14], and in rat embryos in cells in the ventral tegmental area as well as in cells in the developing forebrain [2]. In mice, CCK expression was identified in olfactory sensory neurons during development and shown to play a role in regulating GnRH-1 neuronal migration from nasal regions into the forebrain [6]. The purpose of this study was to map CCK transcript expression in the mouse brain throughout embryonic development. All mice were sacrificed following adequate measures to minimize pain or discomfort. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. NIH Swiss Embryos were harvested at embryonic day (E)12.5, 13.5, 14.5 and E17.5 (plug day, E0.5), immediately frozen and stored (–80°C) until sectioning. In a similar manner, adult brains were frozen and stored (–80°C) until processing. Section from embryos and adult brains were cryosectioned at 16 µm. Serial sections were obtained which were processed as described below.

Three adult CD-1 female mice (2–6 months old; Charles River, Milan, Italy) were anesthetized with an ip injection of ketamine (200 mg/kg), and perfused with 4% paraformaldehyde. The brains were dissected and postfixed in the same fixative overnight at 4°C, cryoprotected in sucrose solutions and sectioned on a cryostat (30 µm thick free-floating sections). Coronal sections were cut from olfactory bulb to the level of the median eminence. Three additional adult CD-1 female mice (2–6 months old; Charles River, Milan, Italy) were treated with colchicine to enhance visualization of CCK immunoreactivity in cell bodies [3]. Colchicine treatment was performed as follows: 10 µg colchicine (Sigma) in 1.5 µl saline, was injected into the lateral ventricle (stereotaxic coordinates: 0 mm bregma line, 1 mm lateral to sagittal sinus, and 2 mm depth) by means of a glass micropipette and a pneumatic pressure injection apparatus (Picospritzer II, General Valve, Fairfield, IL) under xylazine/ketamine anesthesia (5mg/Kg xylazine + 75mg/Kg ketamine). After the micropipette was removed, the skin was sutured and the mice quickly revived under a warm lamp. 72 h after the treatment, animals were sacrificed as described above.

In situ hybridization was performed as previously described [29]. Three different 48 nucleotide oligo probes (5 pmol), complementary to the coding region of mouse pre-proCCK were designed. Probe 1 for CCK was: 5-GGACCTGCT-GGATGTATCGGGCTAGCAGTGC GCCCAGGCGCGCTCGGG–3; probe 2: 5-GCC-AGAGGGAGCTTTGCGGACCTGCTGGATGTATCGGGCTAGCAGTGC–3 and probe 3: 5-GGTCTGGGAGTCACTGAAGGAAACACTGCCTTCCGACCACACAGCTAG–3. The probes were 3' end-labeled with ³⁵S dATP (specific activity 1000–1500 Ci/mmol: Dupont-NEN), 100 U terminal deoxynucleotidyl transferase (Boehringer Mannheim), and 5X tailing buffer (Gibco BRL, Grand Island, NY) to a specific activity of 10,000–18,000 Ci/mmol. Labeled probe (1,000,000 cpm) was applied to each slide in 50 µl of hybridization buffer. All anti-sense probes gave similar labeling patterns. Sense strand controls were negative for all sections analyzed.

Primary rabbit polyclonal antisera used were against: GnRH-1 (SW 1:3000; Dr. Wray, NINDS, NIH, Bethesda, MD, USA or LR5 1:4000; Dr. Robert Benoit, University of Montreal, Quebec),

CCK-8 (1:1000; ImmunoStar Inc, Hudson, Wisconsin; anti-CCK-8, Chemicon), neurophysin (1:1500; Dr. A Robinson), LH (1:1500; Cambridge Medical Diagnostic, Billerica, MA, USA) and GH (1:4000; Dr. AF. Parlow, NIDDK, NIH, Bethesda, MD, USA). Briefly, fresh frozen sections (16 μ m) were fixed (4% formalin, 1 hr), washed (PBS), blocked (10% NGS /0.3% triton X-100 (1 hr), washed several times in PBS, and then incubated in primary antibody overnight at 4°C. The next day, sections were washed (PBS), incubated (1 hr) in biotinylated secondary antibody (1:500 in PBS/0.3 % triton X-100; GAR-Bt -Vector Lab, CA; GAM-Bt -Chemicon) and processed for avidin-biotin-horseradish peroxidase/3'-3-diaminobenzidine (DAB) histochemistry.

To determine the expression pattern of CCK during brain development, *in situ* hybridization (ISHH) was performed using a synthetic deoxynucleotide against pre-proCCK mRNA in embryos as well as in adult mice. Here we present a description of the expression pattern of CCK mRNA and show that CCK expression occurs in several neuronal areas throughout development (Table 1).

Starting at E12.5, robust CCK expression was detected in the spinal cord, metencephalon, myelencephalon, thalamic areas and in the primordium of the anterior pituitary and these signals were upregulated by E14.5 (Fig. 1) and maintained throughout embryonic development (Table 1). At E17.5, CCK mRNA was distributed in more CNS regions (Fig. 2, Table 1). These regions included the frontal and pyriform cortex (Fig. 2A, D, E). A robust signal was also detected in areas such as the cingulate cortex (Fig. 2A–C), the peri- and paraventricular nuclei (PVN; Fig. 2A), the hippocampus (Fig. 2F–H), the mammillary bodies (Fig. 2I–K) and the cuneate nucleus (Fig. 2 L–N). In addition at E17.5, CCK expression was now detected in the median eminence (ME, Fig. 2A, L–N) and the signal in the anterior pituitary was restricted to cells directly adjacent to the ME (Fig. 2L–N).

The ME is the neurovascular link between the hypothalamus and the anterior lobe of the pituitary where axons of the neuroendocrine hypophysiotropic systems terminate. In addition, it is the region through which, primarily oxytocin (OT) and vasopressin (VP), axons pass on route to the posterior lobe of the pituitary. By E14.5 in mice, GnRH-1 neurons reach the forebrain and their axons start to innervate the ME [16]. Thus, either pre-proCCK mRNA was being expressed by support cells within the ME or was expressed in GnRH-1 cells and/or other neuroendocrine cells, likely residing in the peri-or paraventricular nucleus, and being transported to their axon terminals within the ME. To address this issue, the pattern of protein expression of CCK, GnRH-1, neurophysin (a marker of both OT and VP cells), luteinizing hormone and growth hormone (LH and GH, markers for anterior pituitary cell types) was determined by immunohistochemistry and relative positions mapped with respect to the location of CCK mRNA (Fig. 3). For this analysis, immunohistochemistry and ISHH were conducted on adjacent sections from the same animal at E17.5. Using an antibody directed against CCK-8 peptide we confirmed our ISHH data, with CCK staining being segregated in the external but not in the internal zone of the ME (Fig. 3B). GnRH-1 immunoreactive axon terminals were distributed, as expected, in the external zone of the ME where CCK mRNA and protein were localized (Fig. 3C). At this stage, GnRH-1 fibers just started to insinuate themselves in the ME and only single axon or small groups of axons are present at this level [18]. In agreement with other studies [12,26,30], GnRH-1 projections into the pituitary were not observed during the embryonic periods examined instead immunoreactive GnRH-1 fibers stopped at the level of the median eminence. Figures 3D and 3E show a clear topographic segregation of neurophysin immunoreactivity, with the neurophysin stained terminals distributed dorsally to the CCK/GnRH-1 labeled zone. Immunohistochemistry for LH revealed the absence of the anterior lobe of the pituitary in our sections (Fig. 3E). LH has been shown to be a good marker of pars tuberalis as well anterior lobe proper [11,19], strengthening the evidence that GnRH-1 and CCK (both peptide and transcript) were indeed localized in the ME.

However to confirm ME localization, immunohistochemistry for GnRH-1, CCK and GH was performed on E17.5 consecutive sections, 16 μ m apart from each other (Fig. 3F, coronal sections 1, 2 and 3). GH is known to be an early marker of the anterior lobe of the pituitary (prior to LH) and its expression in the anterior pituitary has been shown to dramatically increase from E15.5 to E17.5 during mouse embryonic development [11]. Co-expression of GnRH-1 and CCK immunoreactivity was found, with a distribution similar to that seen in Figs. 1A–C, whereas no GH was detected in this tissue. In agreement with previous studies [11], a very intense GH signal was instead detected in more caudal sections containing the anterior pituitary (Fig. G, coronal section 13).

In situ hybridization performed in adult mice revealed that pre-proCCK mRNA expression in the ME was still present (Fig. 3H). Since colchicine is often required to detect CCK peptide in cell soma *in vivo*, i.c.v. colchicine injections were performed on 3 adult female mice (Fig. 3H upper right insets). The lack of GnRH-1-immunoreactive varicosities in the median eminence (ME) in colchicine-treated animals confirms the effectiveness of the treatment. As a matter of fact, by inhibiting microtubule polymerization, colchicine prevented the neuropeptide translocation to the axon terminals, thus depleting the ME of GnRH-1 immunoreactivity. Following colchicine treatment, a small group of CCK-immunoreactive cell bodies were detected in the ME (see arrows). In addition, strongly labeled CCK-immunoreactive cell bodies were detected in the PVN and periventricular regions [7], areas known to contain cells that send axons to the ME.

In summary, this report details, by ISHH, the distribution of CCK in mouse brain during embryonic development. We document that CCK mRNA is expressed prenatally in multiple areas of the CNS, many of which maintain CCK mRNA expression postnatally into adult life. We also report the localization of CCK mRNA in the ME and a corresponding signal in cells in the anterior pituitary. The relation of these two CCK expressing cell populations is unclear. CCK expression in the developing ME may be associated with cells residing in this region, or it may reside in fibers belonging to hypothalamic neuroendocrine cell types projecting to the external layer of the ME.

CCK is a neuropeptide abundantly expressed in the brain postnatally [27] and has been implicated in both reproductive [10,22,25] as well as non-reproductive behaviors [1]. CCK interacts with two G-protein coupled receptors CCK-1R and CCK-2R [20], CCK-1R has been identified in orexin-producing hypothalamic neurons [25] and in prenatal GnRH-1 cells [6,7]. Prenatally a functional role for CCK on GnRH-1 cell movement was identified, inhibiting cell migration via the CCK-1 receptor signal transduction pathway. The expression pattern of CCK mRNA reported in this paper suggests that CCK may be an important developmental modulator during brain embryogenesis.

CCK has been shown to be a modulator of neuroendocrine pathways, activating female reproductive behaviors and release of anterior pituitary hormones [10,28]. CCK levels in several brain nuclei (medial preoptic nucleus, bed nucleus of the stria terminalis and medial nucleus of the amygdala) appear to be regulated by circulating gonadal steroids [21] and a variety of postnatal *in vivo* studies have been conducted on the role of CCK on GnRH-1 release (10, 22). Although the functional significance of axonal CCK mRNA in the ME is unknown, other regulatory peptide mRNAs such as preprogalanin mRNA [13] and vasopressin mRNA [24] have been previously described in the axonal compartment of hypothalamo-hypophyseal system. This phenomenon could allow a rapid local regulation of the production of proteins in response to different stimulations [17,23].

Acknowledgment

This work was supported, in part, by the Intramural Research Program of the NINDS, NIH. P.G. was partly supported by Ricerca Scientifica Applicata CIPE-A23 Reg. Piemonte, Italy and by University of Turin, Italy.

References

1. Beinfeld MC. An introduction to neuronal cholecystokinin. *Peptides* 2001;22:1197–1200. [PubMed: 11457511]
2. Cho HJ, Shiotani Y, Shiosaka S, Inagaki S, Kubota Y, Kiyama H, Umegaki K, Tateishi K, Hashimura E, Hamaoka T, Tohyama M. Ontogeny of cholecystokinin-8-containing neuron system of the rat: an immunohistochemical analysis. I. Forebrain and upper brainstem. *J Comp Neurol* 1983;218:25–41. [PubMed: 6309919]
3. Ciofi P. Phenotypical segregation among female rat hypothalamic gonadotropin-releasing hormone neurons as revealed by the sexually dimorphic coexpression of cholecystokinin and neurotensin. *Neuroscience* 2000;99:133–147. [PubMed: 10924958]
4. Crawley JN, Corwin RL. Biological actions of cholecystokinin. *Peptides* 1994;15:731–755. [PubMed: 7937354]
5. Fride E. Endocannabinoids in the central nervous system: from neuronal networks to behavior. *Curr Drug Targets CNS Neurol Disord* 2005;4:633–642. [PubMed: 16375681]
6. Giacobini P, Kopin AS, Beart PM, Mercer LD, Fasolo A, Wray S. Cholecystokinin modulates migration of gonadotropin-releasing hormone-1 neurons. *J Neurosci* 2004;24:4737–4748. [PubMed: 15152034]
7. Giacobini P, Wray S. Cholecystokinin directly inhibits neuronal activity of primary gonadotropin-releasing hormone cells through cholecystokinin-1 receptor. *Endocrinology* 2007;148:63–71. [PubMed: 17023535]
8. Gulpinar MA, Yegen BC. The physiology of learning and memory: role of peptides and stress. *Curr Protein Pept Sci* 2004;5:457–473. [PubMed: 15581416]
9. Hadjiivanova C, Belcheva S, Belcheva I. Cholecystokinin and learning and memory processes. *Acta Physiol Pharmacol Bulg* 2003;27:83–88. [PubMed: 14570153]
10. Hashimoto R, Kimura F. Inhibition of gonadotropin secretion induced by cholecystokinin implants in the medial preoptic area by the dopamine receptor blocker, pimozide, in the rat. *Neuroendocrinology* 1986;42:32–37. [PubMed: 3079892]
11. Japon MA, Rubinstein M, Low MJ. In situ hybridization analysis of anterior pituitary hormone gene expression during fetal mouse development. *J Histochem Cytochem* 1994;42:1117–1125. [PubMed: 8027530]
12. Jennes L. Prenatal development of the gonadotropin-releasing hormone-containing systems in rat brain. *Brain Res* 1989;482:97–108. [PubMed: 2650804]
13. Landry M, Hokfelt T. Subcellular localization of preprogalanin messenger RNA in perikarya and axons of hypothalamo-posthypophyseal magnocellular neurons: an in situ hybridization study. *Neuroscience* 1998;84:897–912. [PubMed: 9579792]
14. Lay JM, Gillespie PJ, Samuelson LC. Murine prenatal expression of cholecystokinin in neural crest, enteric neurons, and enteroendocrine cells. *Dev Dyn* 1999;216:190–200. [PubMed: 10536058]
15. Levine JE. New concepts of the neuroendocrine regulation of gonadotropin surges in rats. *Biol Reprod* 1997;56:293–302. [PubMed: 9116124]
16. Livne I, Gibson MJ, Silverman AJ. Biochemical differentiation and intercellular interactions of migratory gonadotropin-releasing hormone (GnRH) cells in the mouse. *Dev Biol* 1993;159:643–656. [PubMed: 8405686]
17. Miyashiro K, Dichter M, Eberwine J. On the nature and differential distribution of mRNAs in hippocampal neurites: implications for neuronal functioning. *Proc Natl Acad Sci U S A* 1994;91:10800–10804. [PubMed: 7971965]
18. Monroe BG, Paull WK. Ultrastructural changes in the hypothalamus during development and hypothalamic activity: the median eminence. *Prog Brain Res* 1974;41:185–208. [PubMed: 4614312]

19. Nemeskeri A, Setalo G, Halasz B. Ontogenesis of the three parts of the fetal rat adenohypophysis. A detailed immunohistochemical analysis. *Neuroendocrinology* 1988;48:534–543. [PubMed: 2469029]
20. Noble F, Wank SA, Crawley JN, Bradwejn J, Seroogy KB, Hamon M, Roques BP. International Union of Pharmacology. XXI, Structure, distribution, and functions of cholecystokinin receptors. *Pharmacol Rev* 1999;51:745–781. [PubMed: 10581329]
21. Oro AE, Simerly RB, Swanson LW. Estrous cycle variations in levels of cholecystokinin immunoreactivity within cells of three interconnected sexually dimorphic forebrain nuclei. Evidence for a regulatory role for estrogen. *Neuroendocrinology* 1988;47:225–235. [PubMed: 3362304]
22. Perera AD, Verbalis JG, Mikuma N, Majumdar SS, Plant TM. Cholecystokinin stimulates gonadotropin-releasing hormone release in the monkey (*Macaca mulatta*). *Endocrinology* 1993;132:1723–1728. [PubMed: 8462472]
23. Steward O, Banker GA. Getting the message from the gene to the synapse: sorting and intracellular transport of RNA in neurons. *Trends Neurosci* 1992;15:180–186. [PubMed: 1377425]
24. Trembleau A, Morales M, Bloom FE. Differential compartmentalization of vasopressin messenger RNA and neuropeptide within the rat hypothalamo-neurohypophysial axonal tracts: light and electron microscopic evidence. *Neuroscience* 1996;70:113–125. [PubMed: 8848117]
25. Tsujino N, Yamanaka A, Ichiki K, Muraki Y, Kilduff TS, Yagami K, Takahashi S, Goto K, Sakurai T. Cholecystokinin activates orexin/hypocretin neurons through the cholecystokinin A receptor. *J Neurosci* 2005;25:7459–7469. [PubMed: 16093397]
26. Ugrumov MV, Ivanova IP, Mitskevich MS, Liposits Z, Setalo G, Flerko B. Axovascular relationships in developing median eminence of perinatal rats with special reference to luteinizing hormone-releasing hormone projections. *Neuroscience* 1985;16:897–906. [PubMed: 3912679]
27. Vanderhaeghen JJ, Signeau JC, Gepts W. New peptide in the vertebrate CNS reacting with antigestrin antibodies. *Nature* 1975;257:604–605. [PubMed: 1165787]
28. Vijayan E, Samson WK, McCann SM. In vivo and in vitro effects of cholecystokinin on gonadotropin, prolactin, growth hormone and thyrotropin release in the rat. *Brain Res* 1979;172:295–302. [PubMed: 466475]
29. Wray S, Kusano K, Gainer H. Maintenance of LHRH and oxytocin neurons in slice explants cultured in serum-free media: effects of tetrodotoxin on gene expression. *Neuroendocrinology* 1991;54:327–339. [PubMed: 1758575]
30. Wu TJ, Gibson MJ, Rogers MC, Silverman AJ. New observations on the development of the gonadotropin-releasing hormone system in the mouse. *J Neurobiol* 1997;33:983–998. [PubMed: 9407018]

Fig. 1.

In situ hybridization histochemistry (ISHH) for CCK at E14.5. (A) Autoradiograph of a sagittal E14.5 mouse section. Strong CCK expression is seen in the spinal cord (spc), thalamus (th) and developing pituitary (pit). (B–C) High magnification photomicrograph of the presumptive pituitary. CCK transcript is found in the anterior region of the pituitary (B, dashed line). (D–F) Detail of CCK expression in the developing spinal cord. (G–I) CCK staining in the developing thalamus. Scale bars: (A) = 200 μm; (B, C) = 50 μm; (D, G) = 100 μm; (E, F, H, I) = 30 μm.

Fig. 2.

ISHH for CCK at E17.5. (A) Autoradiography of coronal brain section showing a strong CCK signal in the frontal cortex (fcx), in the cingulate cortex (ccx), PVN (para and periventricular nucleus), the median eminence (me) and pyriform cortex (pcx). (B–Q) Photomicrographs of individual regions exhibiting CCK mRNA. (B–C) Cingulate cortex, (D–E) pyriform cortex, (F–H) hippocampus (hi), (I–K) mammillary body (mb), (L–N) median eminence and anterior pituitary (me, pit), (O–Q) cuneate nucleus (cn). A–E are coronal sections while F–Q are parasagittal sections. Olfactory bulb (ob), cerebellum (cb). Scale bar: (A) = 500 μ m; (B, C) = 100 μ m; (D, E) = 50 μ m; (F) = 600 μ m; (I, O) = 400 μ m; (L) = 200 μ m; (G, H, J, K, M, N, P, Q) = 100 μ m.

Fig. 3.

CCK expression in the median eminence. Comparison of the distribution of CCK transcript (A) and CCK (B), GnRH-1 (C), neurophysin (D) and lutenizing hormone (LH; E) proteins, respectively, in the median eminence (ME) on 5 consecutive E17.5 coronal mouse sections. (A) CCK transcript is robustly expressed in the ME. A similar distribution pattern was observed for both CCK (B) and GnRH-1, suggesting CCK is localized in the external layer (ext. l.) of the ME. (D) Immunohistochemistry for neurophysin. Immunoreactive terminals are distributed in the internal layer (int. l.) of the ME. (E, left panel) Consecutive sections shown in A, C and D were superimposed and pseudocolors attributed to GnRH-1 (green), neurophysin (blue) and CCK (red). CCK and GnRH-1 expressions are limited to the external zone of the ME as opposed to neurophysin which shows a clear topographic segregation in the internal layer of the ME. (E, right panel). Immunohistochemistry for LH revealed the absence of the anterior lobe of the

pituitary in these sections. (F) Immunohistochemistry for GnRH-1, CCK and GH on three consecutive coronal sections (cor.1, 2 and 3) of an E17.5 mouse embryo. As expected, few immunoreactive GnRH-1 terminals have started to insinuate in the external layer (ext. l.) of the ME (cor. 1). CCK immunostaining is detectable in the same regions (cor. 2), whereas labeling for GH revealed the lack of pituitary tissue in these sections (cor. 3). (G) Immunohistochemistry for GH performed in the same brain as F, 160 μm more caudally (cor. 13), in a section containing the anterior lobe of the pituitary confirmed the specificity of the antibody for GH. Arrows indicate a strong GH expression in the pituitary (Pit). (H) ISHH experiment for CCK, conducted on a coronal adult mouse brain section, showed that CCK transcript was still present in the ME (see lower inset in H). In addition, CCK mRNA is robustly expressed in adult brain areas previously described such as cortex (cx), hippocampus (hi) and thalamus. The two upper insets show GnRH-1 and CCK immunoreactivity in the ME of colchicine-treated mice. As expected, in the treated mice no GnRH-1 immunostained terminals is evident at this level, supporting the effectiveness of the treatment. A small group of CCK-positive cells are still evident in the ME following colchicine injection. Scale bar: (A–F) = 40 μm ; (G) = 140 μm ; (H) = 35 μm ; (insets) = 70 μm .

Table 1**CCK Gene Expression in Embryonic CNS**

	E12.5	E13.5	E14.5	E17.5
Cortex				+
Cerebellum (Purkinje cell layer)				+
Pyramidal Cortex				+
Cingulate Cortex				+
Cuneate Nucleus				+
Hippocampus				+
Raphe Nucleus				+
Thalamus			+	+
Subthalamic Nucleus			+	+
Anterior Hypothalamus			+	+
Arcuate Nucleus				+
Paraventricular Nucleus				+
Periventricular Nucleus				+
Posterior Hypothalamus				+
Anterior Amygdala			+	+
Superior Colliculus			+	+
Inferior Colliculus (tectal epithelial region)				+
Mammillary Body				+
Median Eminence			+	+
Pituitary (tegmental neuroepithelium)	+	+	+	+
Medial Geniculate				+
Preectum		+	+	+
Trigeminal Ganglion	+	+	+	+
Vestibular Nucleus				+
Metencephalon	+	+	+	+
Myelencephalon	+	+	+	+
Spinal Cord	+	+	+	+