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Quantitative Autoradiographic Localization of Cholecystokinin Receptors in Rat and Guinea Pig Brain Using ^{125}I-Bolton-Hunter-CCK8

DEBRA L. NIEHOFF[1]

Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL 60064

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NIEHOFF, D. L. *Quantitative autoradiographic localization of cholecystokinin receptors in rat and guinea pig brain using ^{125}I-Bolton-Hunter-CCK8*. PEPTIDES **10**(2) 265–274, 1989.—The autoradiographic localization of receptors for the brain-gut peptide cholecystokinin (CCK) has shown differences in receptor distribution between rat and guinea pig brain. However the full anatomical extent of the differences has not been determined quantitatively. In the present study, ^{125}I-Bolton-Hunter-CCK8 (^{125}I-BH-CCK8) was employed in a comparative quantitative autoradiographic analysis of the distribution of CCK receptors in these two species. The pharmacological profile of ^{125}I-BH-CCK8 binding in guinea pig forebrain sections was comparable to those previously reported for rat and human. Statistically significant differences in receptor binding between rat and guinea pig occurred in olfactory bulb, caudate-putamen, amygdala, several cortical areas, ventromedial hypothalamus, cerebellum, and a number of midbrain and brainstem nuclei. The results of this study confirm the presence of extensive species-specific variation in the distribution of CCK receptors, suggesting possible differences in the physiological roles of this peptide in different mammalian species.

Neuropeptides CCK CCK receptors Quantitative autoradiography Species-specific receptor distribution

THE peptide cholecystokinin (CCK), originally identified in endocrine cells of the gut (33,50), has also been shown to exist in the CNS, principally as the sulfated octapeptide fragment (14, 15, 20, 33, 48, 50, 55, 59). Especially high levels of CCK have been observed by radioimmunoassay in the cerebral cortex and limbic system (4,5). Immunohistochemical studies have also identified CCK-containing cells in these brain areas, as well as in sensory systems, hippocampus, olfactory bulb, and several midbrain nuclei (7, 16, 18, 26, 27, 38, 51, 54, 60, 63). The peptide is colocalized with dopamine (29,30) in the mesolimbic projection originating in the ventral tegmental nucleus, as well as with the inhibitory transmitter GABA in pyramidal cells of the hippocampus (38). CCK is thought to act as a neurotransmitter/neuroregulator in the brain [(15), see also (37, 46, 58) for reviews]. In particular, the colocalization of this peptide with dopamine and GABA suggests that it may function as a modulator of these transmitters, an hypothesis supported by electrophysiological (47, 64, 66) and behavioral (10) data. There is evidence to support a role for CCK in the induction of satiety (1, 2, 9, 12, 25), in the regulation of sensory processes such as nociception (34,71), in memory (8, 19, 35, 36), and in anticonvulsant activity (72).

Receptors for CCK have been studied in mouse, rat, guinea pig, monkey, and human brain, using both membrane homogenate assays and receptor autoradiography, and a variety of radioligands, including ^{125}I-BH-CCK33 (24, 32, 61, 68, 70), ^{3}H-pentagastrin (23), ^{3}H-CCK8 (67), ^{125}I-BH-CCK8 (11, 13, 24, 42, 44, 57), and ^{3}H-propionyl CCK8 (62). Autoradiographic studies have shown a correspondence of receptor and peptide distribution in some brain areas, such as cerebral cortex.

Receptor autoradiography has also identified species differences in the distribution of CCK receptors, particularly between rat and guinea pig (44, 62, 70). For example, while guinea pig cerebellum contains a high density of CCK receptors, rat cerebellum is nearly devoid of receptors. Conversely, CCK receptors are found in rat, but not guinea pig, interpeduncular nucleus. However, a thorough quantitative autoradiographic comparison of CCK receptor distribution and density in rat and guinea pig has not been reported to date.

Because accurate autoradiographic quantification is facilitated by the use of iodinated, rather than tritiated ligands (40), and because the predominant form of CCK in the brain is the octapeptide fragment, I sought to quantitate CCK receptor distribution in rat and guinea pig brain using ^{125}I-BH-CCK8. In addition, as the binding characteristics of this radioligand have been reported in rat (24) and human (13), but not in guinea pig, I studied the binding of ^{125}I-BH-CCK8 to forebrain sections from

[1]Present address: Biotext, 24 Riverdale Road, Yardley, PA 19067.

this species. The density of CCK receptors in over fifty regions of rat and guinea pig brain, as well as the pharmacological characteristics of ^{125}I-BH-CCK8 binding in guinea pig forebrain sections are reported below.

METHOD

Tissue Preparation

Adult male guinea pigs or Sprague-Dawley rats were anesthetized with pentobarbital (50 mg/kg IP) and perfused intracardially with 0.1% formalin in phosphate buffered saline containing 5% (w/v) sucrose. Brains were removed, blocked, mounted on object holders using bovine brain mash, and frozen in liquid nitrogen. Ten μm sections were cut on a cryostat microtome, thaw-mounted on chrome alum-gelatin coated slides, and air dried. Dried sections were stored at −20°C until use.

Pharmacological Characterization in Guinea Pig Forebrain Sections

Prior to autoradiographic studies, the binding characteristics of ^{125}I-BH-CCK8 were determined in guinea pig brain sections. Association and dissociation rates, as well as affinity constants for the radioligand and several related peptides, were determined.

Thawed sections of guinea pig forebrain [+1.7 to +0.2 mm relative to bregma (53)] were preincubated for 30 min at room temperature in 50 mM Tris HCl, pH 7.4, containing 130 mM NaCl, 4.7 mM KCl, 5 mM $MgCl_2$, and 1 mM EGTA (= "Tris saline buffer") plus 0.5% BSA. Sections were subsequently incubated for 150 min at room temperature in Tris saline buffer (pH 6.5) containing 0.025% bacitracin, 1 mM dithiothreitol, 2 μg/ml chymostatin, 4 μg/ml leupeptin, and ^{125}I-BH-CCK8. In preliminary experiments, BSA was shown to inhibit up to 60% of the specific binding (data not shown) and hence was omitted from this incubation medium. HPLC analysis indicated that there was no degradation of the radioligand under these conditions in the BSA-free medium containing protease inhibitors. Less than 5% of the total radioligand concentration was depleted during the incubation period. Blanks were generated by the concomitant addition of 1 μM nonradiolabeled sulfated CCK8 (CCK8-S). Nonspecific binding averaged 7% of total binding. Competition studies were performed by incubating sections with 100 pM ^{125}I-BH-CCK8 and increasing concentrations of CCK-related peptides. Following incubation, sections were washed six times for 15 min each at 4°C in preincubation buffer (containing BSA), dipped briefly in ice-cold water, then wiped from the slide using a glass fiber filter. Radioactivity was determined in a gamma counter at 75% efficiency.

Autoradiography

Sections from seven rostral-caudal levels of both rat and guinea pig brain (n = 3) were labeled with 100 pM ^{125}I-BH-CCK8 as described above. Serial sections were coincubated with 1 μM CCK8-S to generate blanks. At the end of the washing procedure, sections were dipped briefly in ice-cold water to remove buffer salts and BSA and then dried, first under a stream of cold air, followed by transfer to a desiccator. Labeled sections were apposed to LKB ^{3}H-Ultrofilm for 7 days. Autoradiographic standards were prepared by incubating 10 μm sections of bovine brain paste with increasing concentrations of ^{125}I-Bolton-Hunter reagent (1×10^3 dpm/mg protein-1.5×10^5 dpm/mg protein) in 0.1 M phosphate buffer, pH 8.0 for 1 hr at 4°C followed by drying. Standards were exposed along with the labeled sections. Following exposure, the standard sections were solubilized from the slides in 50 μl 1 N NaOH for radioactivity determination by gamma counting and protein determination by the method of Lowry (43).

The concentration of ligand binding was assessed using a Spatial Data computer-assisted image analysis system. The system was calibrated using the standards and an algorithm based on the equation

$$Z = \frac{X^P \times Z_{max}}{X^P + K^P} \text{ where } Z = \text{the digitized gray value}$$

assigned by the computer, Z_{max} the maximum permissible Z value (255 in our case), X = exposure, K is a constant describing the inflection point of the curve, and P a constant describing its steepness (45). Images representing specific binding were produced by digital subtraction of blank images (in the presence of 1 μM CCK8-S) from total images (in the absence of CCK8-S); these reconstructed images were used for all quantitative measurements of receptor density. Receptor densities were statistically compared between rat and guinea pig brain using a two-tailed *t*-test.

Materials

^{125}I-BH-CCK8 (2200 Ci/mmol) and ^{125}I-Bolton-Hunter reagent (2000 Ci/mmol) were obtained from New England Nuclear (North Billerica, MA). Chymostatin, bacitracin, leupeptin, and dithiothreitol were obtained from Sigma (St. Louis, MO), and CCK-related peptides from Peptide Institute, Inc. (Osaka, Japan).

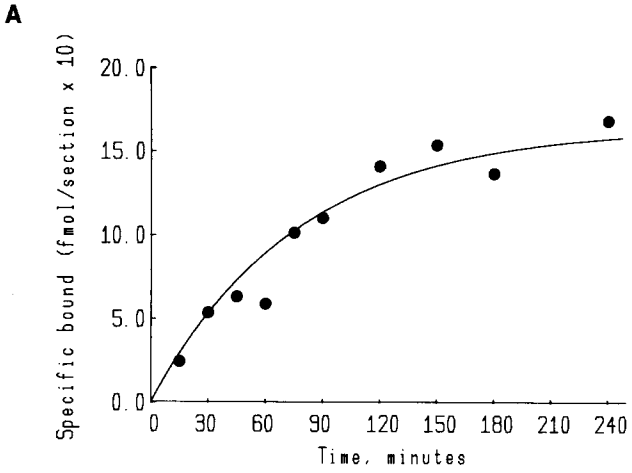
RESULTS

Pharmacological Characterization in Guinea Pig Sections

The association rate of ^{125}I-BH-CCK8 to guinea pig forebrain sections was studied by incubating the sections with 100 pM ^{125}I-BH-CCK8 as described for periods of time up to 4 hr. As illustrated in Fig. 1, specific binding reached equilibrium by 2.5 hr. This time was chosen for future binding studies. The mean value (± S.E.M.) for K_{obs} from 4 experiments was 0.0165 ± 0.0020 min^{-1} and K_1 was calculated as 1.25×10^8 M^{-1} min^{-1}.

The dissociation rate was determined by incubating sections with 100 pM ^{125}I-BH-CCK8 for 2.5 hr, followed by infinite dilution in preincubation buffer for 0–180 min. Nonspecific binding diminished rapidly (Fig. 1B), but specific binding declined quite slowly in a monophasic fashion over this time period. In 4 experiments, K_{-1} was determined to be 0.0040 ± 0.0003 min^{-1} (mean ± S.E.M.). In additional experiments (data not shown), multiple washes (up to six of 15 min each) further reduced the nonspecific binding without significantly affecting specific binding.

Saturation studies were performed using concentrations of ^{125}I-BH-CCK8 in the range of 1–600 pM. Scatchard transforms of the resulting binding isotherms were linear. A mean (± S.E.M.) K_D of 92.77 ± 8.00 pM and B_{max} of 3.82 ± 0.35 fmol/section (about 25 fmol/mg protein; n = 3; Fig. 2) were determined graphically. Competition experiments were performed by coincubating sections with 100 pM ^{125}I-BH-CCK8 and increasing concentrations of CCK8-S, desulfated CCK8 (CCK8-DS), gastrin, CCK4, or the physiologically inactive peptide fragment Boc-CCK3. The rank order of potency for these peptides was CCK8(S) > CCK8(DS) = gastrin > CCK4 >> Boc-CCK3. K_i values for the physiologically active fragments were derived by computer analysis of the competition curves, using a one-site model. Mean values (± S.E.M.) were: 0.085 ± 0.028 nM (CCK8-S); 2.43 ± 0.45 nM (CCK8-DS); 1.50 ± 0.01 nM (gastrin); and 25.07 ± 3.6 nM (CCK4) (n = 3, Fig. 3). No radioligand displacement was observed with Boc-CCK3 at



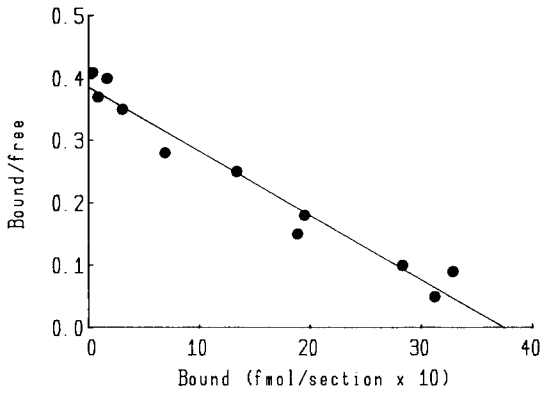


FIG. 2. Scatchard analysis of ^{125}I-BH-CCK8 binding to guinea pig forebrain sections. A single representative experiment is shown, which was repeated twice with similar results.

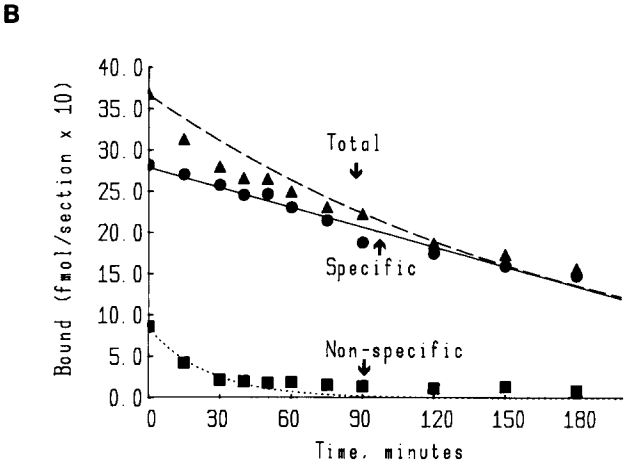


FIG. 1. Association (A) and dissociation (B) of ^{125}I-BH-CCK8 binding to guinea pig forebrain sections. A single representative experiment is shown in each panel, which was repeated three times with comparable results.

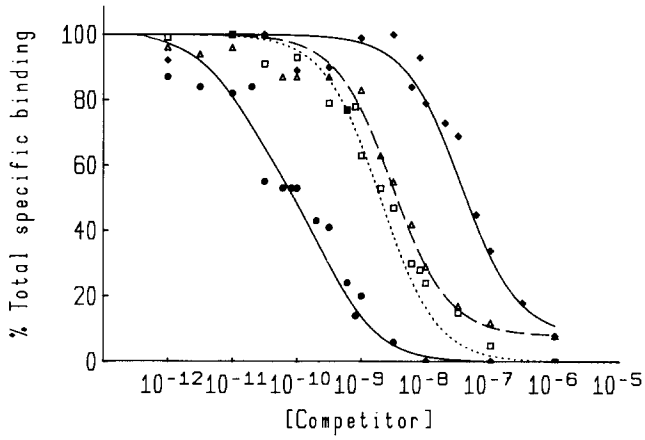


FIG. 3. Competition for ^{125}I-BH-CCK8 binding by CCK-related peptides. (●) CCK8-S; (□) CCK8-DS; (△) gastrin; (◆) CCK4. The results of a single competition experiment are shown for each peptide; each experiment was repeated twice with similar results.

concentrations of up to 1 μM (data not shown).

Radioligand affinity, maximal binding capacity, and the affinity of CCK8-S and CCK4 were also examined in one experiment in rat forebrain sections under identical experimental conditions. The values obtained did not differ substantially from those obtained in the guinea pig.

Comparative Receptor Mapping in Rat and Guinea Pig

Anatomical nomenclature used in describing the distribution of CCK receptors is taken from the atlas of Paxinos and Watson (53). Quantitative data are summarized in Table 1.

Rhinencephalon. Within the guinea pig olfactory bulb, very high levels of binding were observed in the external plexiform layer, and somewhat lower levels in the internal granular layer and glomerular layer (Fig. 4A). The density of binding in the external plexiform layer was the highest observed in guinea pig brain. The same pattern (external plexiform layer > internal granular and glomerular layers) was observed in rat (Fig. 4B), although the absolute receptor density in both layers was significantly lower in this species. In both species, the olfactory tubercle (Fig. 4C, D) and primary olfactory (piriform) cortex (Fig. 4C–F) were rela-

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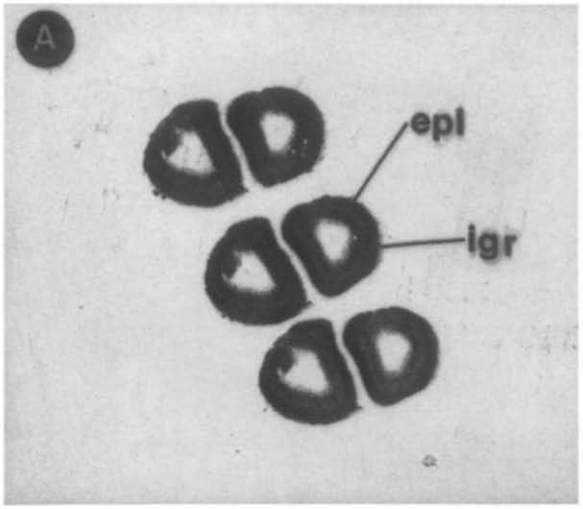
FIG. 4. Autoradiographic localization of ^{125}I-BH-CCK8 in guinea pig (A, C, E) and rat (B, D, F) brain. Photographs are taken directly from ^{3}H-Ultrofilm images at +6.7 mm (A, B); +1.2 mm (C, D); and +0.20 mm (E, F) relative to bregma (45). epl = olfactory bulb, external plexiform layer; igr = olfactory bulb, internal granular layer; cpu = caudate-putamen; po = primary olfactory (piriform) cortex; tu = olfactory tubercle; acb = n. accumbens; VI = isocortex layer VI; cg = cingulate cortex; bst = bed n., stria terminalis. Magnification = 5×.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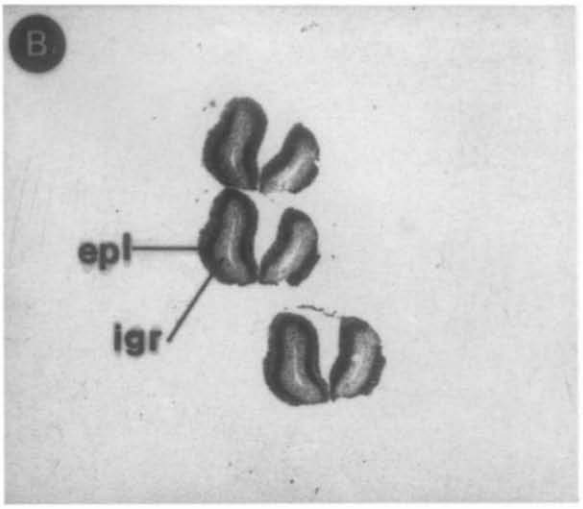
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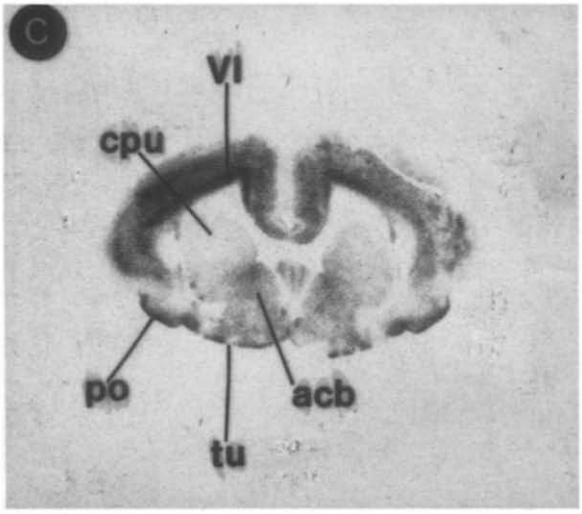
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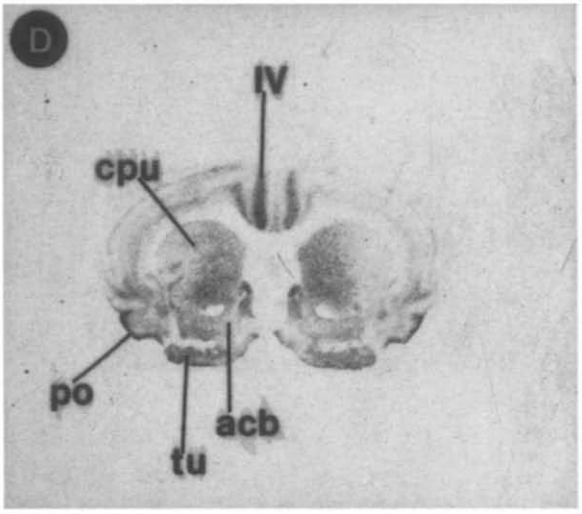
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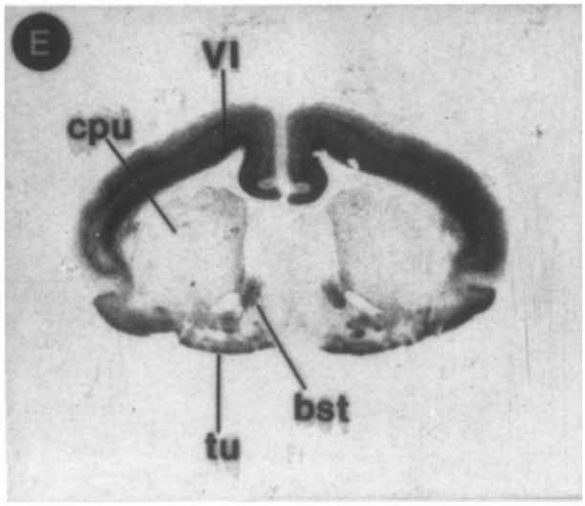
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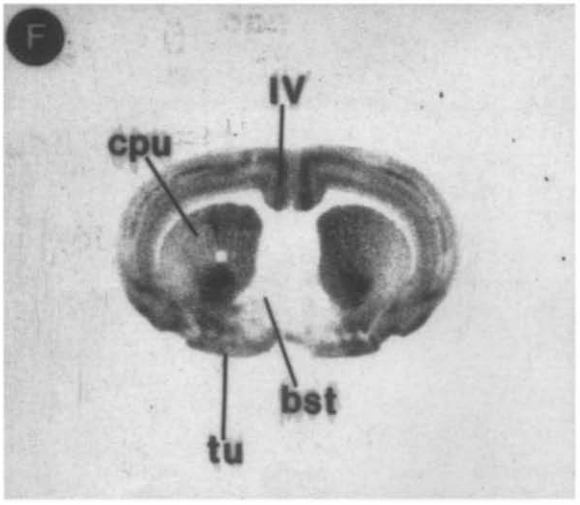
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TABLE 1

DENSITY OF CCK RECEPTORS IN RAT AND GUINEA PIG BRAIN*

Region	Rat	Guinea Pig
Rhinencephalon		
Olfactory bulb		
—external plexiform layer	9.54 ± 0.21‡	24.22 ± 1.13
—internal granular layer	5.85 ± 0.25‡	15.83 ± 0.82
Olfactory tubercle	7.93 ± 0.39†	6.15 ± 0.48
Primary olfactory cortex	7.01 ± 0.03	7.52 ± 0.54
Telencephalon		
Allocortex		
—cingulate cortex, III/IV	11.44 ± 1.42	10.17 ± 0.40
—cingulate cortex, VI	8.25 ± 0.60†	12.11 ± 0.85
—retrosplenial cortex, III/IV	17.56 ± 0.25‡	5.92 ± 0.37
—retrosplenial cortex, VI	10.83 ± 0.34‡	7.07 ± 0.29
—29D, III/IV	17.75 ± 0.54‡	9.55 ± 0.17
—29D, VI	11.68 ± 0.67	9.85 ± 1.23
—AIP, VI	9.45 ± 0.70	7.52 ± 1.60
Isocortex		
—FR1, IV	12.86	14.96 ± 2.12
—FR1, VI	8.04 ± 0.54‡	16.70 ± 1.42
—FR2, IV	5.87 ± 0.57†	11.62 ± 1.75
—FR2, VI	5.78 ± 0.54‡	16.21 ± 1.59
—FL, IV	5.31 ± 0.62†	11.62 ± 1.75
—FL, VI	5.20 ± 0.38‡	16.21 ± 1.59
—HL, IV	9.66	15.86 ± 2.05
—HL, VI	7.87 ± 0.72‡	17.15 ± 0.75
—Par1, VI	4.77 ± 0.33‡	18.05 ± 1.89
—OclM, IV	13.88 ± 1.37	11.49 ± 0.69
—OclM, VI	11.98 ± 0.83	10.68 ± 0.59
Basal ganglia		
—caudate-putamen, medial	6.92 ± 0.76‡	3.00 ± 0.20
—caudate-putamen, lateral	3.64 ± 0.27	2.87 ± 0.13
—globus pallidus	1.16 ± 0.17	2.29 ± 0.32
Septal area		
—n.accumbens, dorsomedial	10.69	8.73 ± 0.30
—n.accumbens, ventrolateral	8.97	7.23 ± 0.44
Amygdala		
—lateral n.	8.05 ± 1.32	9.37 ± 0.93
—central n.	2.87 ± 0.21	3.30 ± 0.31
—medial n.	8.36 ± 0.88†	4.16 ± 0.42
—anterior cortical n.	7.80 ± 0.44	6.66 ± 1.22
—posterior cortical n.	11.51 ± 1.47	8.09 ± 0.72
—bed n., stria terminalis	1.84 ± 0.31	6.15
Hippocampus		
—CA4	4.06 ± 0.21‡	8.24 ± 0.87
—dentate gyrus	3.12 ± 0.54	5.31 ± 1.06
Diencephalon		
Thalamus		
—reticular n.	3.87 ± 0.42	3.71 ± 0.54
—zona incerta	2.10 ± 0.19	3.37 ± 0.55
Hypothalamus		
—ventromedial n.	11.36 ± 1.63‡	1.59 ± 0.11
Mesencephalon		
Ventral tegmental area	0.63 ± 0.04‡	1.60 ± 0.21
Substantia nigra	1.50 ± 0.07	1.09 ± 0.18
Superior colliculus	2.30 ± 0.21†	6.04 ± 1.22
Inferior colliculus, dorsomedial	3.60 ± 0.63	2.50 ± 0.40
Inferior colliculus, ventrolateral	2.60 ± 0.29	1.51 ± 0.22
Ventral lateral geniculate	1.54 ± 0.11	4.46
Medial geniculate	0.70 ± 0.09‡	2.76 ± 0.23
N. Darkschewitsch	0.95 ± 0.14†	2.89 ± 0.66
Brain stem		
Cerebellum, molecular layer	0.26 ± 0.03‡	8.16 ± 0.63
Cerebellum, granular layer	0.47 ± 0.10‡	11.52 ± 1.33
Dorsal tegmental n.	0.92 ± 0.05†	2.36 ± 0.51
Locus coeruleus	1.17 ± 0.07	1.79 ± 0.22
Dorsal parabrachial n.	1.81 ± 0.07	1.97 ± 0.25
Ventral parabrachial n.	1.50 ± 0.13	1.65 ± 0.34
N. solitary tract	4.64 ± 0.80	4.08 ± 0.86
Area postrema	5.20	9.75
N. spinal tract of V	1.75 ± 0.17‡	2.83 ± 0.35

*All data expressed as fmol/mg protein (mean ± S.E.M.) for 3 animals or as the mean of 2 animals varying by <10%.

†$p<0.05$, ‡$p<0.01$.

tively enriched in receptors. The olfactory tubercle displayed a significantly higher density of CCK receptors in the rat.

Telencephalon. In the septal region (Fig. 4C, D) the highest density of CCK receptors was observed in both species in the nucleus accumbens, with the density in the dorsomedial aspect being slightly elevated compared to the ventrolateral aspect. In both species, the medial septal nucleus contained a low level of binding sites, while receptor density in the lateral septum was nearly equal to blank values (Fig. 4C–F).

CCK receptor densities in the basal ganglia were appreciable only in the caudate-putamen, and this nucleus displayed a striking difference in distribution pattern between rat and guinea pig. In the rat caudate, a pronounced medial-lateral gradient in the density of receptors was observed (Fig. 4D, F), with the medial aspect containing nearly two times the density of receptors measured in the lateral aspect. No such gradient was observed in the guinea pig (Fig. 4C, E). In this species, binding in the medial and lateral caudate were equivalent, with the exception of a slight elevation in

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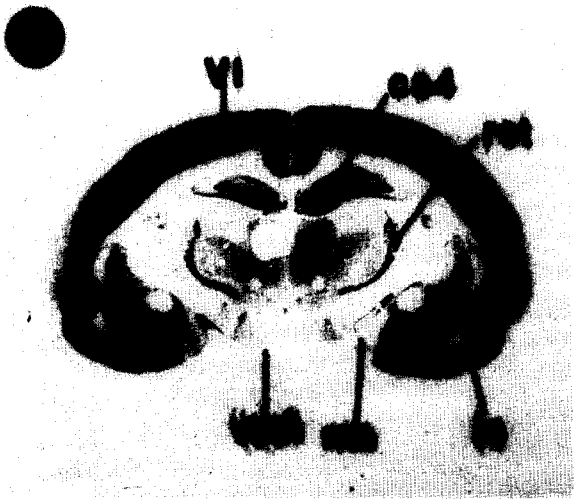
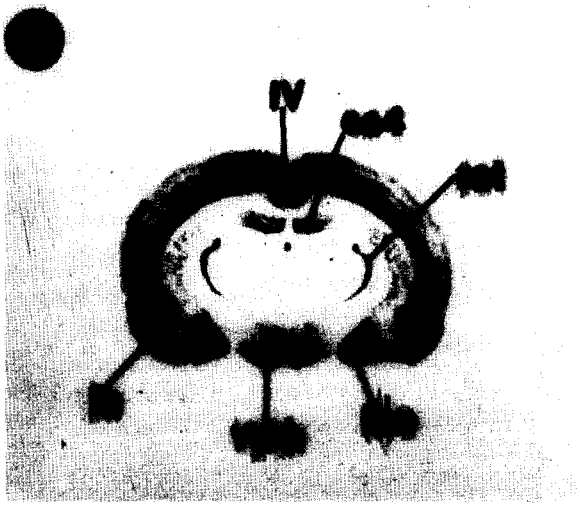
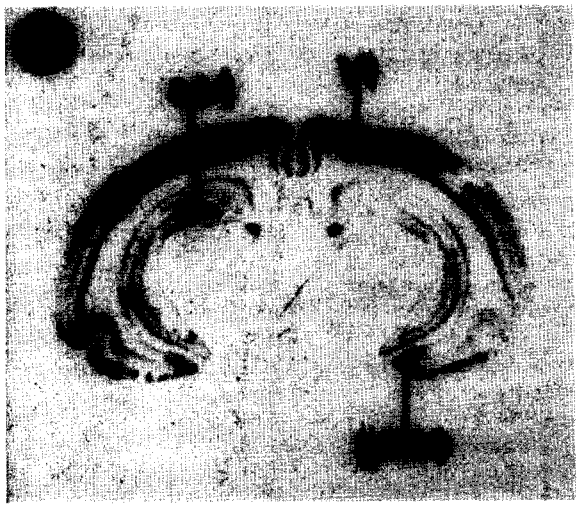
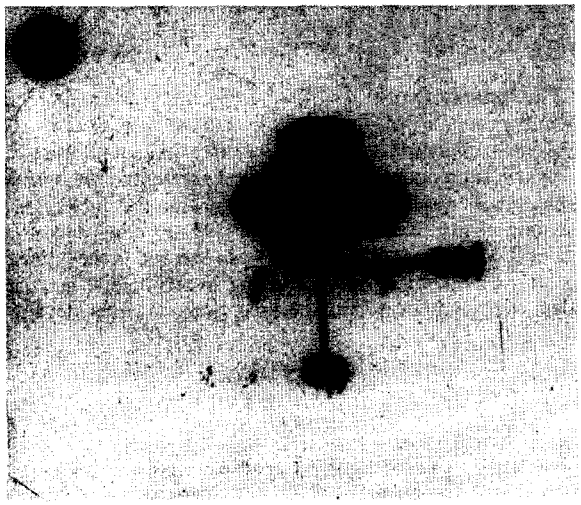
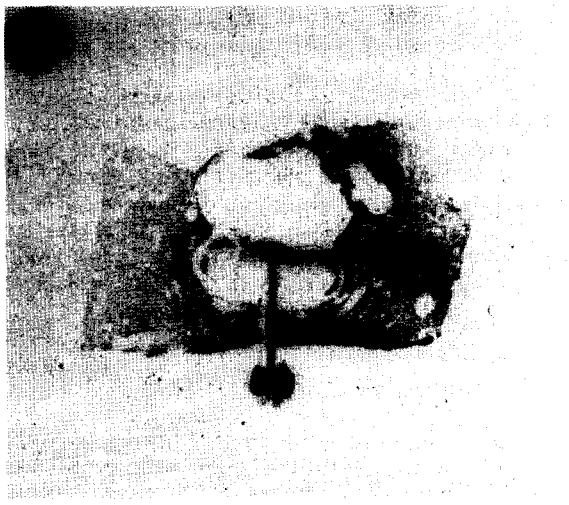
FIG. 5. Autoradiographic localization of ^{125}I-BH-CCK8 in guinea pig (A, C, E) and rat (B, D, F) brain at −2.8 mm (A, B); −4.8 mm (C, D); and −13.8 mm (E, F) relative to bregma (45). Photographs were generated as described in Fig. 4. ret = reticular n. of thalamus; me = medial n. of amygdala; la = lateral n. of amygdala; pmco = posterior cortical n. of amygdala; vmh = ventromedial hypothalamus; rspl = retrosplenial cortex; sol = n. of the solitary tract; ap = area postrema; other abbreviations as in Fig. 4. Magnification 3.5×.











receptor density within a very narrow band located periventricularly.

The hippocampus contained a moderate CCK receptor density in both species. The area with the highest density was CA4 (Fig. 5A–D); this region contained significantly more receptors in the guinea pig. Somewhat lower levels (comparable in both species) of receptors were observed in the dentate gyrus, while still lower levels occurred in CA1 where they appeared to be associated with the pyramidal cell layer. Area CA3 contained the lowest receptor density in both rat and guinea pig. High levels of CCK receptors were also observed in the presubiculum and parasubiculum, with the density in rat being greater than that in guinea pig (data not shown). Receptor levels in the subiculum of both species were much lower.

Within the amygdala, there were some species-specific differences in subnuclear CCK receptor distribution. In rat, the posterior cortical nucleus had the highest receptor density, with substantial levels of binding also observed in the lateral, medial, and anterior cortical nuclei (Fig. 5B, D). Low levels of receptors were found in the basolateral, basomedial, and especially central nucleus of the rat. In the guinea pig, the posterior cortical nucleus and lateral nucleus were also enriched in receptors, although a lower receptor density was present in the posterior cortical nucleus compared to the rat. The central nucleus was also the area of lowest density in the guinea pig amygdala. However, the medial nucleus of this species contained low to moderate amounts of receptors, comparable to the basolateral, basomedial, and central nuclei (Fig. 5A, C). In the bed nucleus of the stria terminalis, guinea pig receptor density was approximately three times that observed in the rat (Fig. 4E, F).

The cerebral cortex was, in general, an area of high CCK receptor density. Both rat and guinea pig displayed a high density of receptors in the cingulate cortex. These receptors were principally located in intermediate layers (III and IV) in the rat, while in the guinea pig it was layer VI that was particularly enriched (Fig. 4C–F). This species-specific laminar pattern of binding was preserved in more caudal regions of allocortex, such as retrosplenial cortex (Fig. 5A–D). In addition, the concentration of receptors was significantly greater in rat retrosplenial cortex than in guinea pig; indeed, this area contained one of the highest densities of receptors in the rat brain. Within frontal isocortex (FR1 and FR2), higher levels of CCK binding were observed in the guinea pig. Frontal cortical receptors in this species were concentrated in layers IV and especially VI, with lower levels in other laminae (Fig. 4C, E). In the same cortical areas of the rat, binding was approximately equivalent in layers IV and VI, with moderate levels of CCK receptors also occurring in more superficial laminae (Fig. 4D, F). In parietal cortex (FL, HL, and Par1), CCK receptor density was greatly elevated in guinea pig compared to rat. In both species, more medial aspects (FL and HL) contained CCK receptors in layers IV and VI in approximately equal numbers, while in more lateral aspects (Par1), layer VI was particularly enriched in receptors (Fig. 4C–F; Fig. 5A, B). Finally, within occipital cortex, receptor densities were approximately equivalent in rat and guinea pig, with binding in layer IV slightly greater than that in layer VI (Fig. 5C, D).

Diencephalon. For the most part, CCK receptor densities in the diencephalon of both rat and guinea pig were fairly low. With the exception of the paraventricular nucleus in the rat and reticular nucleus of both species (both of which contained only moderate amounts of receptors; Fig. 5A–B), receptor density in the thalamus was very low. The guinea pig hypothalamus was similarly an area of low receptor density (Fig. 5A). However, in the rat, very high levels of CCK receptors were observed in the ventromedial nucleus of the hypothalamus (Fig. 5B) and a high receptor density was also observed in the supraoptic nucleus (data not shown).

Mesencephalon. At mesencephalic levels, modest CCK receptor densities were observed in both species in the inferior colliculus, where the dorsomedial division contained more receptors than the ventromedial division. The guinea pig displayed significantly enriched receptor binding in the superior colliculus; this was confined to the superficial gray layer. The guinea pig brain also contained modest levels of CCK receptor binding in both medial and ventral lateral geniculate nuclei, as well as in the nucleus of Darkschewitsch; these were all areas of very low receptor density in the rat. Surprisingly low receptor densities were observed for both species in the substantia nigra/ventral tegmental area (Fig. 5C, D), in spite of the known electrophysiological actions of CCK in this region (47,64).

Brainstem. One of the more striking differences in CCK receptor distribution between the two species was observed in the cerebellum. Although this region contained nearly blank levels of receptor binding in the rat, a high receptor density was observed in the guinea pig. Binding in the guinea pig was somewhat higher in the granular layer compared to the molecular layer, while binding in white matter was extremely low (Fig. 5E, F).

Most other areas of the brain stem contained very low receptor densities in both rat and guinea pig. Two exceptions were the nucleus of the solitary tract and the area postrema (Fig. 5E, F). In guinea pig, the area postrema had about twice the receptor density found in the nucleus of the solitary tract, while in rat, the two regions were comparable. Lower levels of receptor binding were observed in the cuneate nucleus (Fig. 5E, F), dorsal tegmental nucleus, locus coeruleus, and dorsal and ventral parabrachial nuclei. Receptor binding in the guinea pig was slightly higher than in the rat in all of these regions.

DISCUSSION

The binding of ^{125}I-BH-CCK8 to guinea pig forebrain sections was saturable, of high affinity, and displaced at appropriate concentrations of physiologically active CCK-related peptides. Thus, the binding sites labeled by this ligand in guinea pig are likely to correspond to a physiologically relevant CCK receptor. Moreover, the rank order of potency of the various peptides examined in competition studies indicates that these binding sites possess a pharmacological profile similar to that described by others (32) for CCK receptors in the brain rather than those found in the periphery. In both kinetic and equilibrium binding studies, ^{125}I-BH-CCK8 appeared to label a single homogeneous population of binding sites with a comparable affinity. Some differences were observed in the binding of ^{125}I-BH-CCK8 to receptors in sections compared to results obtained in guinea pig cortical membranes (42). For example, the affinity of the radioligand was lower in membranes, as were the affinities of CCK8-S, CCK8-DS, and CCK4. Some evidence for receptor heterogeneity was also observed in the guinea pig cortical membrane preparation (42). These differences may be due to variations in the assay conditions utilized, or they may represent differences in CCK receptors found in the "intact" membranes of tissue sections compared to receptors in the more "disrupted" membranes of homogenate preparations. The results of this study in guinea pig forebrain sections also differed somewhat from those previously reported in rat forebrain sections (24). In particular, the affinity of both CCK8-S and ^{125}I-BH-CCK8 were approximately an order of magnitude higher in the guinea pig. In addition, the affinity of CCK8-NS and CCK4 was lower, while that of gastrin was slightly higher. Variations in assay conditions, especially the omission of BSA in the current study, may account for these differences. In recent studies of ^{125}I-BH-CCK8 binding to human brain sections (11,13), the

observed rank order of potency of CCK8-S, CCK4, and gastrin was similar to that reported here in guinea pig; however, the affinity of all of these peptides was considerably lower in human.

In agreement with earlier studies (24, 44, 62, 70), striking differences in the distribution of CCK receptors were observed between rat and guinea pig. Statistically significant species-specific differences in receptor density were noted in the olfactory bulb, olfactory tubercle, cingulate, retrosplenial, frontal, and parietal cortices, medial caudate-putamen, the medial nucleus of the amygdala, bed nucleus of the stria terminalis, CA4 of the hippocampus, ventromedial hypothalamus, ventral tegmental area, superior colliculus, medial geniculate, nucleus of Darkschewitsch, cerebellum, dorsal tegmental nucleus, area postrema, and caudal nucleus of the spinal trigeminal tract. Thus, the anatomical extent of the difference in receptor localization pattern between these two species is far greater than previously reported. The affinities of ^{125}I-BH-CCK8, CCK8-S, and CCK4 were similar in both species; thus, as argued by other investigators (44), it is unlikely that the species-related differences in receptor binding are due to variations in the binding of the radioligand between rat and guinea pig. Species-specific variations in the distribution of CCK receptors have also been reported in mouse, primate, and human brain (11, 13, 24, 57, 62). For example, in contrast to rat and guinea pig brain, CCK receptors are absent in mouse caudate-putamen, and present in very low levels in nucleus accumbens [(62), Dietl and Palacios, submitted]. Similarly, CCK receptor density is comparatively low in mouse hippocampus, superior colliculus, and thalamic reticular nucleus. Moderate levels of CCK receptors are present in mouse cerebellum, resembling the guinea pig rather than the rat brain. However, whereas the receptor density is higher in the granular layer of guinea pig cerebellum, in mouse cerebellum the receptors are concentrated exclusively in the molecular layer (Dietl and Palacios, submitted). The distribution of CCK receptors in primate brain has many similarities to the distribution in guinea pig brain. For example, a moderate to high density of CCK receptors is found in the cerebellum, and these receptors are concentrated in the granular layer (Dietl and Palacios, submitted). In primate neocortex, as in guinea pig cortex, lamina VI is enriched in receptors, while both species contain low levels of receptors in the hypothalamus and in most thalamic nuclei. However, in contrast to guinea and rat, primate brain contains a high density of CCK receptors in superficial layers of the neocortex, as well as a moderate density in the substantia nigra [(24), Dietl and Palacios, submitted]. In human brain, the distribution of CCK receptors is unique in the complete absence of CCK receptors in the olfactory tubercle, as well as in the concentration of receptors in lamina V of the cerebral cortex (13). Like guinea pig and primate, human brain displays a low CCK receptor density in the hypothalamus and thalmus (13,57), and a high density of receptors in the cerebellum, especially in the granular layer (11,13). Human brain also contains a high level of CCK receptors in the Purkinje cell layer. Receptor levels in the human substantia nigra are moderate (11,13), similar to the distribution in primate brain. The exact reason for these extensive species-specific variations in CCK receptor density is not known. Perhaps the differences in receptor distribution reflect variability in the physiological role(s) played by CCK in these species.

Earlier work on the autoradiographic localization of CCK receptors in guinea pig, using ^{125}I-BH-CCK33 (70), described high levels of binding in guinea pig olfactory tubercle, basolateral amygdala, area CA1 of the hippocampus, ventral lateral geniculate, parabrachial nucleus, and dorsal tegmental nucleus and moderate levels of binding in substantia nigra. Using ^{125}I-BH-CCK8, the current study identified only moderate levels of CCK receptors in the olfactory tubercle, and low levels of receptors in the other regions. In addition, while the previous study (70) described binding sites for CCK33 in several thalamic (e.g., anterodorsal nucleus) and brain stem (e.g., parabigeminal and superior olivary nuclei) nuclei, the current study failed to observe receptors in these regions. In guinea pig, therefore ^{125}I-BH-CCK8 may label only a subset of the CCK receptor population labeled by ^{125}I-BH-CCK33, an hypothesis supported by the finding that the maximal binding capacity of this ligand in guinea pig cortical membranes is approximately four times greater than that observed by us in forebrain sections from this species (68).

The localization of CCK in the CNS has been most extensively studied in the rat. In this species at least, there is correspondence between areas of high CCK receptor density and areas of high CCK peptide content. For example, cortical interneurons containing CCK as well as CCK-positive terminals have been identified in layer VI of the cerebral cortex (18, 27, 54), an area of high CCK receptor density. The high level of receptors observed in the ventromedial hypothalamus can be correlated with the existence of CCK afferents from the superior lateral parabrachial nucleus (21, 31, 69), while receptors in the posterior cortical amygdaloid nucleus and in the pyramidal cell layer of the hippocampus correlate with the presence of immunohistochemically defined CCK nerve terminals in these regions (7, 26, 51, 60). Other regions of good CCK receptor-peptide correspondence include the area postrema, nucleus of the solitary tract, dorsal tegmental nucleus, medial and cortical nuclei of the amygdala, caudate-putamen, nucleus accumbens, and supraoptic nucleus. However, areas of noncorrespondence also occur. For example, the amount of CCK found in the central nucleus of the amygdala is one of the highest in the brain, and includes CCK-containing terminals (16, 60, 63), but this region contains levels of CCK receptors close to background. Other regions with CCK-positive cells and/or terminals, but low levels of receptors include many hypothalamic nuclei, ventral tegmental area, substantia nigra, dorsal raphe nucleus, and periaqueductal grey area. Conversely, in guinea pig brain, the cerebellum contains a significant amount of receptor binding, but CCK levels in this structure are extremely low (41). In the hippocampus of this species, there is a high density of CCK-containing fibers (22), but receptor levels are only low to moderate. Possible reasons for such receptor-transmitter mismatches have been reviewed (28,39).

Because of the colocalization of CCK and dopamine in mesolimbic dopaminergic neurons (29,30), as well as data suggesting that CCK can modulate dopaminergic function (10, 47, 64), it is especially interesting to note that several regions of the rat brain which contain high levels of CCK receptors are also projection sites for dopaminergic neurons and contain dopamine receptors. For example, both CCK receptors and dopamine receptors (52) are concentrated in the nucleus accumbens (especially the mediodorsal aspect) and in anterior cingulate cortex. This receptor colocalization is concomitant with the colocalization of CCK and dopamine in neurons projecting to these structures from the ventral tegmental area (29,30), and may represent an anatomical substrate for the observed potentiation of dopamine-induced hyperactivity by CCK following administration within the nucleus accumbens (10). In the rat, CCK receptors are also found at high levels in the caudate, in a medial-lateral gradient pattern consistent with the known topographic pattern of dopaminergic innervation from the medial substantia nigra/ventral tegmental area (17). Although CCK is not colocalized with dopamine in these neurons (29,30), it is possible that receptors for the two substances are found on the same postsynaptic cells and could potentially interact. Such receptor-receptor interaction could account for the observed effects of CCK on the binding of dopamine receptor ligands in the caudate (6,49). Alternatively, CCK receptors localized to presynaptic dopamine terminals could account for the action of CCK on striatal dopamine release (65). Surprisingly, few CCK receptors

are labelled by ^{125}I-BH-CCK8 in the ventral tegmental area, where a high density of cells containing both CCK and dopamine have been demonstrated (29,30) and where the peptide has been shown to potentiate dopamine-mediated inhibition of cell firing rate (47,64). This may indicate that this particular effect of CCK is due to a subset of receptors not labeled by ^{125}I-BH-CCK8.

The potential relationship between CCK receptor distribution and the observed physiological and behavioral actions of this peptide has been discussed by earlier investigators (70). The results of the current study, in concordance with these suggestions, support a role for CCK in feeding behavior and satiety (via receptors in the ventromedial hypothalamus, posterior cortical nucleus of the amygdala, limbic system, cortex, and olfactory areas), sensory processing (via receptors on olfactory visual and auditory areas as well as layer IV of the cortex), and cortical function. Recent evidence has also implicated CCK in anticonvulsant activity (72) and the regulation of memory (8, 19, 35, 36). Potential anatomical loci of these effects could be CCK receptors in the hippocampus or retrosplenial cortex, respectively. The enrichment of CCK receptors in motor areas of cortex (such as FR1 and FR2), and in guinea pig cerebellum, also suggest that the peptide could be important in the regulation of motor behavior.

In summary, the current study confirms, quantitates, and extends previous observations of extensive variation in the distribution of CCK receptors between rat and guinea pig. The pronounced difference in receptor density in many brain regions between these two closely related species serves to highlight the potential problem posed by such species differences in the design of appropriate experimental animal models.

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REFERENCES

1. Antin, J.; Gibbs, J.; Holt, J.; Young, R. C.; Smith, G. P. Cholecystokinin elicits the complete behavioral sequence of satiety in rats. J. Comp. Physiol. Psychol. 89:784–790; 1975.
2. Baile, C. A.; Laughlin, C. L.; Della-Fera, M. A. Role of cholecystokinin and opioid peptides in control of food intake. Physiol. Rev. 66:172–232; 1986.
3. Basso, N.; Bagarini, M.; Gizzonio, D.; Basoli, A.; Fiocca, F.; DePaolis, C.; Praga, C.; Speranza, V. Analgesic effect of ceruletide in biliary and renal colic. Gastroenterology 80:1105; 1981.
4. Beinfeld, M. C.; Meyer, D. K.; Eskay, R. L.; Jensen, R. T.; Brownstein, M. J. The distribution of cholecystokinin immunoreactivity in the central nervous system of the rat as determined by radioimmunoassay. Brain Res. 212:51–57; 1981.
5. Beinfeld, M. C.; Palkovits, M. Distribution of cholecystokinin (CCK) in the hypothalamus and limbic system of the rat. Neuropeptides 2:123–129; 1981.
6. Bhoola, K. D.; Dawbarn, D.; O'Shaughnessy, C.; Pycock, C. J. Modulation of dopamine receptor activation by the neuropeptides VIP and CCK. Br. J. Pharmacol. 77:334P; 1982.
7. Cho, H. J.; Shiotani, Y.; Shiosaka, S.; Inagaki, S.; Kubota, Y.; Kiyama, H.; Umegaki, K.; Tateishi, K.; Hasimura, E.; Hamaska, T.; Tohyama, M. Ontogeny of cholecystokinin-8-containing neuron system of the rat: An immunohistochemical analysis. I. Forebrain and upper brainstem. J. Comp. Neurol. 218:25–41; 1983.
8. Cohen, S. L.; Knight, M.; Tamminga, C. A.; Chase, T. N. Cholecystokinin-octapeptide effects on conditioned-avoidance behavior. Eur. J. Pharmacol. 83:213–222; 1982.
9. Crawley, J. N.; Rojas-Ramirez, J. A.; Mendelson, W. The role of central and peripheral cholecystokinin in mediating appetitive behaviors. Peptides 3:535–538; 1982.
10. Crawley, J. N.; Stivers, J. A.; Blumstein, L. K.; Paul, S. N. Cholecystokinin potentiates dopamine-mediated behaviors: evidence for modulation specific to a site of co-existence. J. Neurosci. 5:1972–1983; 1985.
11. Cross, A. J.; Slater, P.; Skan, W. Characteristics of ^{125}I-Bolton-Hunter labelled cholecystokinin binding in human brain. Neuropeptides 11:73–76; 1988.
12. Della-Fera, M. A.; Baile, C. A. Cholecystokinin octapeptide: continuous picomole injections into the cerebral ventricles of sheep suppress feeding. Science 206:471–473; 1979.
13. Dietl, M. M.; Probst, A.; Palacios, J. M. On the distribution of cholecystokinin receptor binding sites in human brain: an autoradiographic study. Synapse 1:169–183; 1987.
14. Dockray, G. J. Immunochemical evidence of cholecystokinin-like peptides in brain. Nature 264:568–570; 1976.
15. Emson, P. C.; Lee, C. M.; Rehfeld, J. F. Cholecystokinin octapeptide: vesicular localization and calcium dependent release from rat brain in vitro. Life Sci. 26:2157–2163; 1980.
16. Fallon, J. H.; Hicks, R.; Loughlin, S. E. The origin of cholecystokinin terminals in the basal forebrain of the rat: evidence from immunofluorescence and retrograde tracing. Neurosci. Lett. 37: 29–35; 1983.
17. Fallon, J. H.; Moore, R. Y. Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. J. Comp. Neurol. 180:545–580; 1978.
18. Fallon, J. H.; Seroogy, K. B. The distribution and some connections of cholecystokinin neurons in rat brain. Ann. NY Acad. Sci. 448: 121–132; 1985.
19. Fekete, M.; Kadar, T.; Penke, B.; Telegdy, G. Modulation of passive avoidance behavior by cholecystokinin octapeptide in rats. Neuropeptides 1:301–307; 1980.
20. Frey, P. Cholecystokinin octapeptide (CCK 26–33), nonsulfated octapeptide and tetrapeptide (CCK 30–33) in rat brain: analysis by high pressure liquid chromatography (HPLC) and radioimmunoassay (RIA). Neurochem. Int. 5:811–815; 1983.
21. Fulwiler, C. E.; Saper, C. B. Cholecystokinin-immunoreactive innervation of the ventromedial hypothalamus in the rat: possible substrate for autonomic regulation of feeding. Neurosci. Lett. 53:289–296; 1985.
22. Gall, C. The distribution of cholecystokinin-like immunoreactivity in the hippocampal foremation of the guinea pig: Localization in the mossy fibers. Brain Res. 306:73–83; 1984.
23. Gaudreau, P.; Quirion, R.; St.-Pierre, S.; Pert, C. B. Characterization and visualization of cholecystokinin receptors in rat brain using [^{3}H]pentagastrin. Peptides 4:755–762; 1983.
24. Gaudreau, P.; St.-Pierre, S.; Pert, C. B.; Quirion, R. Cholecystokinin receptors in mammalian brain: a comparative characterization and visualization. Ann. NY Acad. Sci. 44:198–219; 1985.
25. Gibbs, J.; Young, R. C.; Smith, G. P. Cholecystokinin decreases food intake in rats. J. Comp. Physiol. Psychol. 84:488–495; 1973.
26. Hendry, S. H. C.; Jones, E. G. Morphology of synapses formed by cholecystokinin-immunoreactive axon terminals in regio superior of rat hippocampus. Neuroscience 16:57–68; 1985.
27. Hendry, S. H. C.; Jones, E. G.; Beinfeld, M. C. Cholecystokinin-immunoreactive neurons in rat and monkey cerebral cortex make symmetric synapses and have intimate associations with blood vessels. Proc. Natl. Acad. Sci. USA 80:2400–2404; 1983.
28. Herkenham, M. Mismatches between neurotransmitter and receptor localizations in brain: observations and implications. Neuroscience 23:1–38; 1987.
29. Hokfelt, T.; Rehfeld, J. F.; Skirboll, L.; Ivemark, B.; Goldstein, M.; Markey, K. Evidence for co-existence of dopamine and CCK in meso-limbic neurones. Nature 285:476–478; 1980.
30. Hokfelt, T.; Skirboll, L.; Rehfeld, J. F.; Goldstein, M.; Markey, K.; Dann, O. A subpopulation of mesencephalic dopamine neurons projecting to limbic areas contains a cholecystokinin-like peptide: evidence from immunohistochemistry combined with retrograde tracing. Neuroscience 5:2093–2124; 1980.
31. Inagaki, S.; Shiotani, Y.; Yamano, M.; Shiosaka, S.; Takago, H.; Tateishi, K.; Hashimura, E.; Hamaoka, T.; Tohyama, M. Distribu-

tion, origin, and fine structure of cholecystokinin-8-like immunoreactive terminals in the nucleus ventromedialis hypothalamus of the rat. J. Neurosci. 4:1289–1299; 1984.
32. Innis, R. B.; Snyder, S. H. Distinct cholecystokinin receptors in brain and pancreas. Proc. Natl. Acad. Sci. USA 77:6917–6921; 1980.
33. Ivy, A. C.; Oldberg, E. Hormone mechanism for gall bladder contraction. Am. J. Physiol. 85:381–383; 1928.
34. Jurna, I.; Zetler, G. Antinociceptive effect of centrally administered caerulein and cholecystokinin octapeptide (CCK-8). Eur. J. Pharmacol. 73:323–331; 1981.
35. Katsuura, G.; Itoh, S. Preventive effect of cholecystokinin octapeptide in experimental amnesia in rats. Peptides 7:105–109; 1986.
36. Katsuura, G.; Itoh, S. Passive avoidance deficit following intracerebroventricular administration of cholecystokinin tetrapeptide amide in rats. Peptides 7:809–814; 1986.
37. Kelly, J. S. Electrophysiology of peptides in the central nervous system. Br. Med. Bull. 38:283–290; 1982.
38. Kosaka, T.; Kosaka, K.; Tateishi, K.; Hamaoka, Y.; Yanaihara, N.; Wu, J.-Y.; Hama, K. GABAergic neurons containing CCK_8-like and/or VIP-like immunoreactivities in the rat hippocampus and dentate gyrus. J. Comp. Neurol. 239:420–430; 1985.
39. Kuhar, M. J. The mismatch problem in receptor mapping studies. Trends Neurosci. 8:190–191; 1985.
40. Kuhar, M. J.; Unnerstall, J. R. Quantitative receptor mapping by autoradiography: some current technical problems. Trends Neurosci. 8:49–53; 1985.
41. Larsson, L. I.; Rehfeld, J. F. Localization and molecular heterogeneity of cholecystokinin in the central and peripheral nervous system. Brain Res. 165:201–218; 1979.
42. Lin, C. W.; Miller, T. Characterization of cholecystokinin receptor sites in guinea pig cortical membranes using [^{125}I]-Bolton-Hunter-cholecystokinin octapeptide. J. Pharmacol. Exp. Ther. 232:775–780; 1985.
43. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol Chem. 193: 265–275; 1951.
44. Mantyh, C. R.; Mantyh, P. W. Differential localization of cholecystokinin-8 binding sites in the rat vs the guinea pig brain. Eur. J. Pharmacol. 113:137–139; 1985.
45. Miller, J. A.; Hoffer, B. J.; Zahniser, N. R. Quantitative autoradiographic analysis of [^{125}I]pindolol binding in rat brain: changes in B-adrenergic receptor density with aging. Soc. Neurosci. Abstr. 12:1470; 1986.
46. Morley, J. E. Minireview. The ascent of cholecystokinin (CCK) from gut to brain. Life Sci. 30:479–493; 1982.
47. Mueller, A. L.; Stittsworth, J. D., Jr.; Brodie, M. S. An in vitro study of the actions of cholecystokinin octapeptide and dopamine on midbrain dopaminergic neurons. Soc. Neurosci. Abstr. 12:232; 1986.
48. Muller, J. E.; Straus, E.; Yalow, R. S. Immunohistochemical localization in rabbit brain of a peptide resembling the COOH-terminal octapeptide of cholecystokinin. Proc. Natl. Acad. Sci. USA 74:3035–3037; 1977.
49. Murphy, R. B.; Schuster, D. I. Modulation of [^{3}H]-dopamine binding by cholecystokinin octapeptide (CCK-8). Peptides 3:539–543; 1982.
50. Mutt, V.; Jorpes, J. E. Structure of porcine cholecystokinin-pancreozymin. I. Cleavage with thrombin and trypsin. Eur. J. Biochem. 6:156–162; 1968.
51. Nunzi, M. G.; Gorio, A.; Milan, F.; Freund, T. F.; Somogyi, P.; Smith, A. D. Cholecystokinin-immunoreactive cells form symmetrical synaptic contacts with pyramidal and nonpyramidal neurons in the hippocampus. J. Comp. Neurol. 237:485–505; 1985.
52. Palacios, J. M.; Niehoff, D. L.; Kuhar, M. J. ^{3}H-spiperone binding sites in brain: autoradiographic localization of multiple receptors. Brain Res. 213:277–289; 1981.
53. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates, second edition. New York: Academic Press; 1986.
54. Peters, A.; Miller, M.; Kimerer, L. M. Cholecystokinin-like immunoreactive neurons in rat cerebral cortex. Neuroscience 8:431–448; 1983.
55. Pinget, M. E.; Straus, E.; Yalow, R. S. Localization of cholecystokinin-like immunoreactivity in isolated nerve terminals. Proc. Natl. Acad. Sci. USA 75:6324–6326; 1978.
56. Praissman, M.; Martinez, P. A.; Saladino, C. F.; Berkowitz, J. M.; Steggles, A. W.; Finkelstein, J. A. Characterization of cholecystokinin binding sites in rat cerebral cortex using a ^{125}I-CCK-8 probe resistant to degradation. J. Neurochem. 40:1406–1413; 1983.
57. Quirion, R.; Csonka, C.; Etienne, P.; Nair, N. P. V.; Robitaille, Y.; Gaudreau, P. Autoradiographic localization of cholecystokinin receptors in human brain. Ann. NY Acad. Sci. 448:624–626; 1985.
58. Rehfeld, J. F. Neuronal cholecystokinin: one or multiple transmitters? J. Neurochem. 44:1–10; 1985.
59. Robberecht, P.; Deschodt-Lanckman, M.; Vanderhaegen, J. J. Demonstration of biological activity of brain gastrin-like peptide material in the human. Its relationship with the COOH-terminal octapeptide of cholecystokinin. Proc. Natl. Acad. Sci. USA 75:524–528; 1978.
60. Roberts, G. W.; Woodhams, P. L.; Polak, J. M.; Crow, T. J. Distribution of neuropeptides in the limbic system of the rat: the amygdaloid complex. Neuroscience 7:99–131; 1982.
61. Saito, A.; Sankaran, H.; Goldfine, I. D.; Williams, J. A. Cholecystokinin receptors in the brain: characterization and distribution. Science 208:1155–1156; 1980.
62. Sekiguchi, R.; Moroji, T. A comparative study on characterization and distribution of cholecystokinin binding sites among the rat, mouse,and guinea pig brain. Brain Res. 399:271–281; 1986.
63. Seroogy, K. B.; Fallon, J. H. Projections of CCK-containing neurons in the substantia nigra-ventral tegmental area and raphe nuclei of the albino rat. Anat. Rec. 208:163A; 1984.
64. Skirboll, L.; Grace, A. A.; Hommer, D. W.; Rehfeld, J.; Goldstein, M.; Hokfelt, T.; Bunney, B. S. Peptide monoamine coexistence: studies of the actions of cholecystokinin-like peptide on the electrical activity of midbrain dopamine neurons. Neuroscience 6:2111—2124; 1981.
65. Starr, M. S. Influence of peptides on ^{3}H-dopamine release from superfused rat striatal slices. Neurochem. Int. 4:233–240; 1982.
66. Stittsworth, J. D., Jr.; Giardina, W. J. Cholecystokinin blocks effects of GABA on hippocampal population spike. Soc. Neurosci. Abstr. 11:743; 1985.
67. Van Dijk, A.; Richards, J. G.; Trzeciak, A.; Gillessen, D.; Mohler, H. Cholecystokinin receptors: Biochemical demonstration and autoradiographic localization in rat brain and pancreas using [^{3}H]cholecystokinin8 as radioligand. J. Neurosci. 4:1021–1033; 1984.
68. Williams, J. A.; Gryson, K. A.; McChesney, D. J. Brain CCK receptors: Species differences in regional distribution and selectivity. Peptides 7:293–296; 1986.
69. Zaborsky, L.; Beinfeld, M. C.; Palkovits, M.; Heimer, L. Brainstem projection to the hypothalamic ventromedial nucleus in the rat: a CCK-containing long ascending pathway. Brain Res. 303:225–231; 1984.
70. Zarbin, M. A.; Innis, R. B.; Wamsley, J. K.; Snyder, S. H.; Kuhar, M. J. Autoradiographic localization of cholecystokinin receptors in rodent brain. J. Neurosci. 3:877–906; 1983.
71. Zetler, G. Analgesia and ptosis caused by caerulein and cholecystokinin octapeptide (CCK-8). Neuropharmacology 19:415–422; 1980.
72. Zetler, G. Anticonvulsant effects of caerulein and cholecystokinin octapeptide compared with those of diazepam. Eur. J. Pharmacol. 65:297–300; 1980.