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Cholecystokinin Receptors in the Brain: Characterization and Distribution

Abstract. *Specific cholecystokinin binding sites in particulate fractions of rat brain were measured with iodine 125-labeled Bolton-Hunter cholecystokinin, a cholecystokinin analog that has full biological activity. Binding was detected in brain regions known to contain immunoreactive cholecystokinin. Binding was saturable, reversible, of high affinity (dissociation constant, 1.7 × 10^{-9} M), and was inhibited by cholecystokinin analogs but not by unrelated hormones.*

Certain polypeptide hormones are present both in endocrine cells of the gastrointestinal tract and in cells of the nervous system (*1*). Cholecystokinin (CCK), originally isolated as a 33 amino acid polypeptide (CCK_{33}) from the small intestine, stimulates both pancreatic exocrine secretion and gallbladder contraction (*2*). The biological activity of this hormone is contained in the carboxyl terminal octapeptide (CCK_8) portion of the molecule. This smaller molecule is also present in the mucosa of the small intestine along with a larger variant, CCK_{39} (*3*, *4*).

Vanderhaeghen *et al.* (*5*) described a polypeptide in the brain that reacts with antibodies to gastrin, a 17 amino acid gastrointestinal hormone that stimulates acid secretion in the stomach. The terminal carboxyl pentapeptide sequences are identical in CCK and gastrin. Although CCK occurs naturally only in the sulfated form, gastrins occur in both sulfated and unsulfated forms. In subsequent studies the gastrinlike immunoreactivity in brains was found to be CCK_8 (*6*). Using antibodies that react to various segments of CCK, Larsson and Rehfeld (*7*) found that the central and peripheral nervous systems of the guinea pig contain molecular components of CCK having gel chromatographic elution coefficients corresponding to those of CCK_{33}, CCK_{12}, CCK_8, and CCK_4. Antiserums with carboxyl terminal specificity react with all these forms of CCK, and through the use of such antiserums immunoreactive material has been found in nerves, fibers, and cell bodies of the brain (*7*, *8*).

If CCK regulates brain functions, then brain cells should possess CCK receptors analogous to those for other polypeptides (*9*). However, due to difficulties in radioactively labeling CCK to high specific activity, brain receptors for the hormone have not been previously described. We have reported that the action of CCK on the exocrine pancreas is initiated by occupancy of a high-affinity CCK receptor (*10*). That study was made possible by a new preparation of radioactive iodine-labeled CCK of high specific activity obtained by the conjugation of ^{125}I-labeled Bolton-Hunter (BH) reagent to CCK_{33} (*11*). This ligand has the full biological activity of CCK as determined by assays of amylase release in isolated pancreatic acini of rats and mice (*11*). In the present study, we used this new ligand to characterize high-affinity CCK binding sites in particulate fractions prepared from rat brain.

When the particulate fraction of rat cerebral cortex (*12*) was incubated with 100 p*M* ^{125}I-labeled BH-CCK at 24°C, binding was half-maximal after 10 minutes and maximal after 60 minutes. A binding plateau was maintained for up to 180 minutes (Fig. 1A). When an excess of CCK_8 (10^{-6}*M*) was added with the label, binding of the tracer was reduced to 70 percent or less (nonspecific binding). Specific binding was reversible, since there was rapid displacement of labeled hormone when 10^{-6}*M* unlabeled CCK_8 was added after 60 minutes of incubation (Fig. 1A).

Unlabeled CCK_{33} competed with ^{125}I-labeled BH-CCK for these binding sites (Fig. 1B). Detectable inhibition of specific binding was seen at 0.3 n*M* CCK_{33}, half-maximal inhibition at 1.0 n*M* CCK_{33}, and maximal inhibition at > 100 n*M* CCK_{33}. Scatchard plots (*13*) of the CCK_{33} binding data were linear, a finding compatible with the presence of a single class of binding sites (Fig. 1C). In four brain-membrane preparations, CCK binding had a dissociation constant of 1.7 ± 0.7 n*M* and a binding capacity of 27.3 ± 5.4 fmole per milligram of protein. Other CCK analogs also inhibited the binding of ^{125}I-labeled BH-CCK. As estimated from the midpoint of parallel displacement curves, CCK_8 was three times more potent than CCK_{33}, desulfated human gastrin was half as potent as CCK_{33}, and desulfated CCK_8 and CCK_4 were one-fourth as potent as CCK_{33} (Fig. 1B). Unrelated peptides including insulin, secretin, pancreatic polypeptide, substance P, and β-endorphin all failed to inhibit binding of ^{125}I-labeled BH-CCK at a concentration of 10^{-7}*M*.

Like the CCK receptors in the brain, those in pancreatic acini have the highest affinity for CCK_8. However, the affinity of the pancreatic receptors is much reduced for analogs without a sulfated tyrosine in position 7 from the carboxyl terminus. Thus the affinity for CCK_8 in rat pancreatic acini is about 5000 times greater than that for either desulfated CCK_8 or gastrin, while the affinity for CCK_4 is at least ten times weaker than that for desulfated CCK (*10*). Accordingly, neither gastrin nor CCK_4 is a potent agonist in rat pancreatic acini. In contrast, the mammalian stomach responds to both gastrin and CCK. Receptors in rat stomach have the highest affinity for gastrin; CCK_{33} is one-half as potent (*14*). Also, in the stomach the

Table 1. Distribution of CCK receptors in rat brain and the regional content of CCK. Rat brains were dissected on ice and the specified regions separately homogenized. Specific binding was determined as described in the legend to Fig. 1, except only 50 p*M* ^{125}I-labeled BH-CCK was used. All values for binding are the mean ± standard error for three experiments. N.A., not assayed.

Brain region	Specific CCK binding (fmole/mg protein × 10^2)	CCK content* (pg/mg, wet weight)
Cerebral cortex	106 ± 13	550
Olfactory bulb	112 ± 3	185
Caudate nucleus	90 ± 13	N.A.
Hippocampus	52 ± 9	103
Hypothalamus	51 ± 15	179
Hindbrain	14 ± 6	45
Midbrain	9 ± 6	N.A.
Cerebellum	0	0

*Data are taken from Schneider *et al.* (*16*).

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presence or absence of a sulfated tyrosine is of little importance for biological activity (*15*). The specificity of the brain receptors is intermediate between that of the stomach and pancreas receptors in that they have the highest affinity for CCK_8 and are also highly reactive with both desulfated CCK_8 and gastrin. It is not surprising, therefore, that the shared carboxyl terminal tetrapeptide (CCK_4 or tetragastrin) also interacts with the brain CCK receptor. Thus the brain CCK receptor is able to interact with at least three of the forms of CCK believed to be present in brain—CCK_{33}, CCK_8, and CCK_4.

Radioimmunoassay measurements have shown that CCK is not equally distributed throughout the rat brain. The highest concentrations are found in the cerebral cortex, with lower levels in the hippocampus, olfactory lobes, caudate nucleus, and hypothalamus. Little CCK is present in the brainstem and even less in the cerebellum (*4, 6, 16*). Table 1 gives the distribution of ^{125}I-labeled BH-CCK binding sites. Although nonspecific binding was similar in all areas of the brain, specific binding was highest in the cerebral cortex, olfactory lobes, and caudate nucleus. Thus there was a correlation between the distribution of CCK in the rat brain and its specific binding sites.

The function of CCK in the brain is unknown. One possible role for CCK is that of a hormone or neurotransmitter mediating satiety. Introduction of food into the intestine induces satiety, and this effect can be mimicked by injecting CCK (*17*). Recently, much smaller doses of CCK_8 were shown to diminish feeding behavior when injected intraventricularly in sheep (*18*). In that study CCK_8 was more potent than pentagastrin. The importance of the CCK_8 molecule is also emphasized by the existence in the brain of a trypsinlike converting enzyme that cleaves CCK_{33} to CCK_8 (*19*). Straus and Yalow (*20*), using a specific carboxyl terminal antibody, reported that the concentration of brain CCK (expressed as CCK_8 equivalents) was reduced in the cerebral cortex of genetically obese mice of the *ob/ob* genotype. However, in a study in which an antibody to desulfated CCK_8 was used, no difference in cortical CCK levels was found between *ob/ob* mice and their normal littermates (*16*). It was also reported that CCK acts as a neurotransmitter in the hippocampus to increase the firing rate of pyramidal neurons (*21*). Further studies on the relations between brain CCK levels, CCK receptors, and CCK action will help explain the nature of polypeptide regulation of brain function.

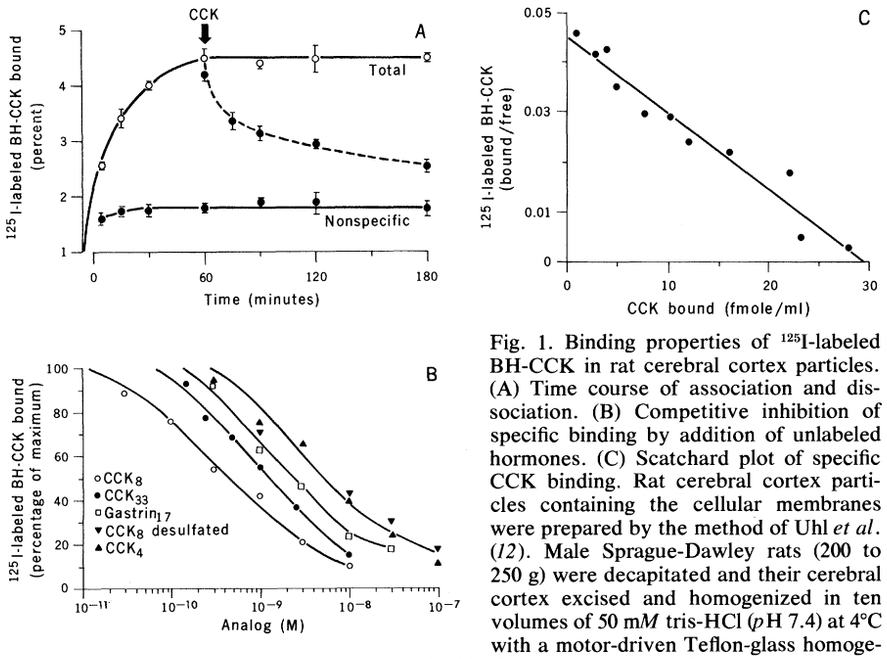

Fig. 1. Binding properties of ^{125}I-labeled BH-CCK in rat cerebral cortex particles. (A) Time course of association and dissociation. (B) Competitive inhibition of specific binding by addition of unlabeled hormones. (C) Scatchard plot of specific CCK binding. Rat cerebral cortex particles containing the cellular membranes were prepared by the method of Uhl *et al.* (*12*). Male Sprague-Dawley rats (200 to 250 g) were decapitated and their cerebral cortex excised and homogenized in ten volumes of 50 m*M* tris-HCl (*p*H 7.4) at 4°C with a motor-driven Teflon-glass homogenizer. The homogenate was centrifuged at 42,000*g* for 15 minutes at 4°C, resuspended in an equal volume of buffer, and centrifuged again. The pellet was then resuspended by homogenization in buffer containing 130 m*M* NaCl, 4.7 m*M* KCl, 5 m*M* $MgCl_2$, 1 m*M* EGTA, 5 mg of bovine serum albumin per milliliter, and 10 m*M* Hepes (*p*H 7.4). To measure binding, ^{125}I-labeled BH-CCK (*11*) was added, at a concentration of 50 to 100 p*M*, to 0.5 ml of membranes (final protein concentration, 0.7 to 1.0 mg/ml) in plastic tubes at 24°C. At specified intervals, duplicate 200-μl portions were withdrawn and centrifuged through 100 μl of ice-cold incubation buffer with a Beckman 152 microcentrifuge. The pellets were rinsed with cold buffer and recentrifuged. Then the tips of the tubes were cut off and the radioactivity was determined. Other hormones were added as specified. Nonspecific binding was determined by adding 10^{-6}*M* CCK_8, and this value was subtracted from total binding to yield specific binding. (A) and (C) show the results of representative experiments: the values in (A) are the mean ± standard error of triplicate determinations; the values in (B) are pooled from eight experiments and normalized as the percentage of specific binding. Lines were fit visually to the data points, with the points for desulfated CCK_8 and CCK_4 fitted by a single curve.

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