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Cholecystokinin and its COOH-terminal octapeptide in the pig brain

(neuropeptides/radioimmunoassay/size and charge discrimination/antibody specificity/gastrointestinal-like peptide)

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ABSTRACT **Two components—one resembling intact cholecystokinin in size and charge and immunologic specificity, and the other resembling the COOH-terminal octapeptide of cholecystokinin—have been found in extracts of the pig cerebral cortex. The relative concentrations of the two peptides in the extracts were dependent on the extractant, boiling 0.1 M HCl being more effective than boiling water for the extraction of intact cholecystokinin but less effective for the extraction of the octapeptide. The physiologic role of these peptides in the brain has yet to be elucidated.**

Much interest has been generated recently in the finding of peptides common to the brain and the gastrointestinal tract. A determination of the location and concentration of these peptides has usually depended on immunologic techniques, either immunocytochemical localization or radioimmunoassay. The finding by Vanderhaeghen *et al.* (1) of a new peptide in the vertebrate central nervous system that reacts with antibodies against gastrin has been confirmed by Dockray (2) who suggested, on the basis of its pattern of immunoactivity with different antisera and its elution pattern on Sephadex G-25, that the brain factor resembled cholecystokinin (CCK)-like peptides more closely than it did gastrin-like peptides. In this report, we describe the identification in the pig brain of two peptides, one resembling intact CCK and the other resembling the COOH-terminal octapeptide of cholecystokinin (CCK-8).

MATERIALS AND METHODS

Gastrin and CCK Peptides. The heptadecapeptide of porcine gastrin (PGI) was a gift from R. Gregory; the sulfated synthetic CCK-octapeptide (CCK-8) was a gift from Squibb Research Institute, NJ, through the courtesy of S. J. Lucania; CCK was purchased from the Gastrointestinal Hormone Research Unit, Karolinska Institute, Stockholm, Sweden; synthetic human [15-leucine]-gastrin I and gastrin tetrapeptide amide [(G-14–17)-] were purchased from Research Plus Laboratories, Denville, NJ.

Preparation of Antisera. An antiserum against intact porcine CCK was prepared by immunization of goat 1 with CCK coupled to bovine serum albumin with carbodiimide (3). This antiserum reacted quite sensitively with purified porcine CCK but reacted poorly with CCK-8. The antiserum also reacted strongly with extracts of the gastrointestinal mucosa from the pig but not from other species. Rabbit B was immunized with G-(14–17)-coupled to bovine serum albumin with carbodiimide. This antiserum reacted most strongly with PGI, less strongly with CCK-8, and quite weakly with intact CCK. The immunization schedule and methodology for antibody production were typical of those generally used in our laboratory (4).

Labeled Antigen. ^{125}I-Labeled synthetic human [15-leucine]-gastrin I and ^{125}I-labeled CCK were prepared by using our minor modification of the chloramine technique using about 1.2 mCi of ^{125}I (Amersham-Searle) for each microgram of gastrin and 0.5 mCi of ^{125}I for each microgram of CCK. Purification was done by using starch gel electrophoresis for labeled gastrin (4) and by using adsorption to and elution from Quso G32 for labeled CCK (4).

Radioimmunoassay. Radioimmunoassay was done with a 2.5-ml incubation volume. The standard diluent was generally 0.02 M barbital, pH 8.6, containing either 0.2 g of bovine serum albumin per 100 ml or 2% fetal bovine serum. The concentration of the labeled gastrin tracer and the labeled CCK tracer was generally <0.5 pg/ml and <10 pg/ml, respectively. The crossreactivities of PGI, intact CCK, and CCK-8 were studied with each of the antisera. Radioimmunoassay of extracts, starch-block eluates, and Sephadex fractions was performed by using methods quite similar to those established in our laboratory for other hormones (4, 5).

Brain Extracts. Immediately after death, specimens for extraction were taken from various portions of the brain of the pig including the cortex, the cerebellum, and the pons. The tissues were sectioned while still frozen, 0.1 M HCl or distilled water was added to produce a concentration of 0.1 g wet weight of tissue per ml, the solutions were boiled for 3 min, and then the tissues were homogenized in their extraction solution by using a Teflon tissue grinder. The extracts were assayed directly with each antiserum and were also fractionated by starch block electrophoresis and on Sephadex columns using radioactive marker molecules to locate the positions of the void volume and the salt peak. These methods were similar to those described from our laboratory for the gastrin peptides (6, 7).

RESULTS

The crossreactivities of PGI, CCK, CCK-8, and water and acid extracts of the pig cerebral cortex were studied with each of the antisera (Fig. 1). The brain extracts crossreacted strongly with the goat antiserum against porcine CCK which had good sensitivity for the detection of porcine CCK but low sensitivity for the detection of PGI and CCK-8; this extract also crossreacted strongly with the rabbit antiserum against G-(14–17)- which had good sensitivity for the detection of PGI and CCK-8 but much poorer sensitivity for the detection of intact CCK. These findings suggest that the brain extract contains both CCK-like and CCK-8-like peptides. No detectable immunoreactivity was observed for the pons or cerebellum extracts with either antiserum. The 0.1 M HCl extract of the cortex appeared to contain 0.4 μg of CCK per g wet weight of tissue by using the goat anti-CCK serum and 0.03 μg of CCK-8 per g wet weight of tissue by using rabbit B antiserum. The boiling water extraction resulted in apparent concentrations of 0.2 μg of CCK per g wet weight of tissue and 0.2 μg of CCK-8 per g wet weight of tissue. Thus, 0.1 M HCl is more efficient for the extraction of the CCK-like peptide, but water is more efficient for the extraction of the CCK-8 like peptide from the brain.

Abbreviations: CCK, cholecystokinin; CCK-8, COOH-terminal octapeptide of cholecystokinin; PGI, heptadecapeptide of porcine, gastrin I; G-(14–17)-, gastrin tetrapeptide amide.

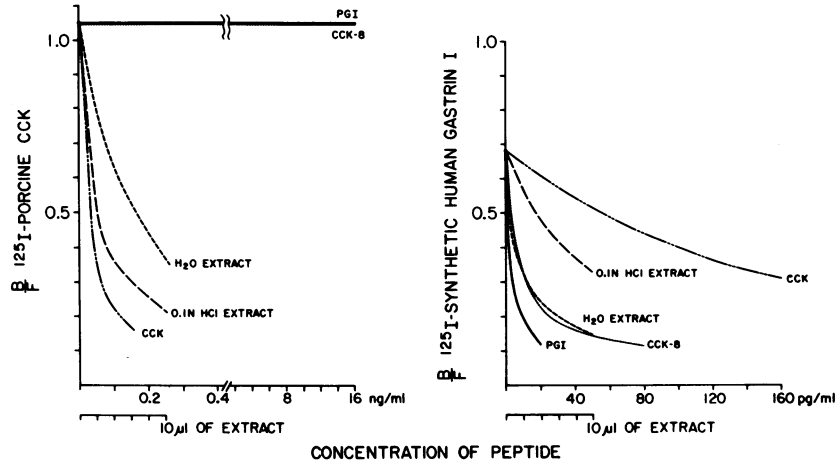

FIG. 1. Crossreactions of PGI, CCK, CCK-8, and water and 0.1 M HCl extracts of the pig cortex shown by their competing against ^{125}I-CCK with goat antiserum against porcine CCK (1:10,000 dilution) (*Left*) and against ^{125}I-labeled synthetic human gastrin I with rabbit antiserum against G-(14–17)- (1:20,000 dilution) (*Right*). B/F, bound/free ratio.

Eluates from Sephadex G-50 gel filtration and starch block electrophoresis were assayed with each of the antisera to characterize further the nature of the peptides in the brain extract. The results obtained with Sephadex fractionation are shown in Fig. 2. When the eluates were assayed with goat 1 antiserum, a major peak of immunoreactivity was detected in the intact CCK region but not in the CCK-8 region; when assayed with rabbit B antiserum, the major peak of immunoreactivity corresponded to that of CCK-8 and there was no detectable immunoreactivity in the CCK region.

It would thus appear that both CCK-like and CCK-8-like peptides are found in the pig cortex and that the ability to observe one or the other of these peptides depends on the specificity of the antiserum employed for the assay.

Similar findings were obtained after starch block electrophoresis. The brain extract immunoreactivity had an electrophoretic mobility resembling CCK when the goat 1 antiserum was used and CCK-8 when assayed with the rabbit B antiserum (Fig. 3).

We conclude that there are at least two immunoreactive components in the pig cortex, one corresponding in size and charge to CCK and the other to CCK-8.

DISCUSSION

The finding of related peptides common to the brain and to the gastrointestinal tract has generated much interest and considerable speculation. The apparent lack of chemical specificity of the opiate receptors in the brain in that alkaloids and small peptides, certainly substances of very different chemical properties, presumably react with the same receptor sites (8) emphasizes that a sterical configuration does not assure a unique chemical structure. Identification of unknown substances by immunologic behavior alone may similarly be beset by problems of specificity. Thus, purification and chemical characterization are ultimately required for absolute identification of biologic constituents. Nonetheless, immunologic reactivity coupled with evaluation of behavior in a variety of chemical or physicochemical systems can provide guidelines for subsequent chemical identification of unknown substances. Thus, studies on the heterogeneity of gastrin, based on radioimmunoassay and chemical and physicochemical characterization of picogram to nanogram amounts of the gastrin-like peptide in plasma or tissue extracts containing a 1×10^6-fold excess of other proteins (7), were predictors of the properties of big gastrin, the 34-amino-acid peptide later purified by Gregory

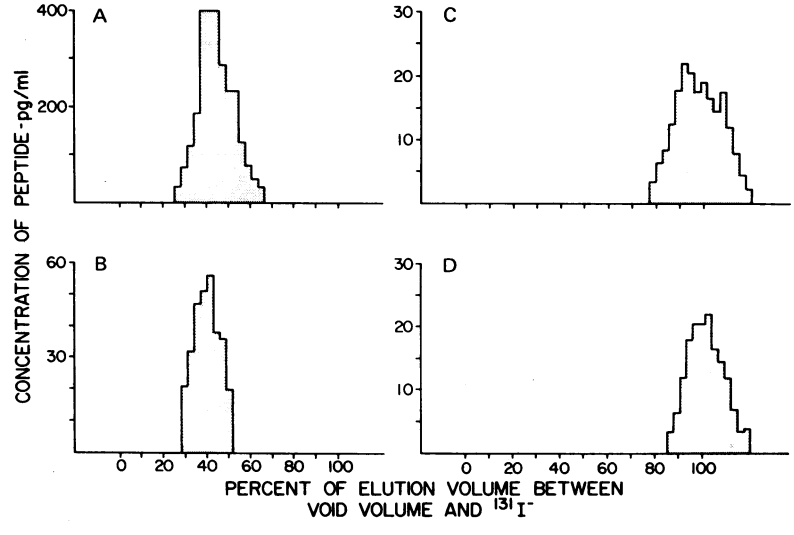

FIG. 2. Concentration of immunoreactivity in eluates after Sephadex G-50 gel filtration of CCK (*A*) or an acid extract of pig cortex (*B*) as measured with goat 1 antiserum or of CCK-8 (*C*) or an acid extract of pig cortex (*D*) as measured with rabbit B antiserum.

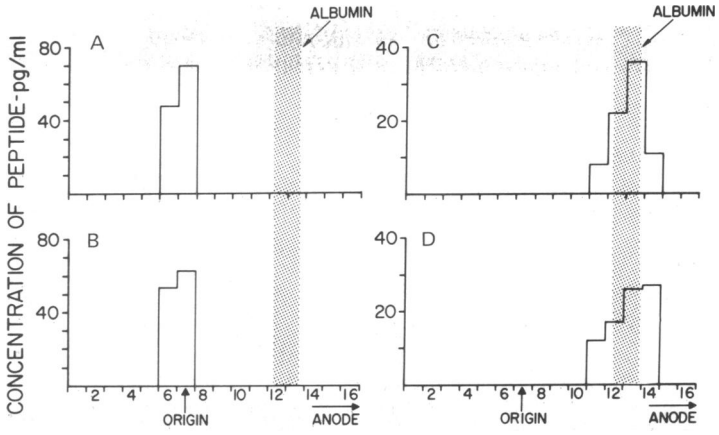

FIG. 3. Concentration of immunoreactive material in eluates after starch block electrophoresis of CCK (*A*) or an acid extract of pig cortex (*B*) as measured with goat 1 antiserum or of CCK-8 (*C*) or an acid extract of pig cortex (*D*) as measured with rabbit B antiserum.

and Tracy from gastrinomas and the gastrointestinal mucosa (9).

The brain peptide first identified by Vanderhaeghen *et al.* (1) by its reaction with antibodies against gastrin was shown to be of smaller molecular weight and distinguishable immunochemically from gastrin-(2–17). The brain peptide resistance to tryptic digestion indicated that, like gastrin, it did not contain lysine or arginine groups. Dockray (2) later suggested that the brain component resembled a CCK-like peptide more closely than a gastrin-like peptide on the basis of its elution volume on Sephadex G-25 and its reactivity with several antisera. However, the data he presented would not have permitted distinction between a COOH-terminal gastrin peptide such as gastrin heptapeptide and a COOH-terminal CCK peptide. The five COOH-terminal amino acids of these two peptides are identical and there is another amino acid, a sulfated tyrosine, which is common to both. Differentiation between these peptides would have been strengthened by further chemical or physicochemical characterization including the use of systems that separate substances on the basis of charge such as starch block (as described in the present report), starch gel, or the use of ion exchange resins. Dockray also stated (2) that the immunoreactive brain components he observed differed from those of previously characterized forms of CCK. The specificity of the particular antisera employed is of course crucial in determining whether or not substances are measurable by radioimmunoassay and this might account for his failure to have detected CCK.

Our demonstration that peptides resembling both intact CCK and its COOH-terminal octapeptide are found in the pig cortex suggests that both are synthesized there and that there is a direct precursor–product relationship between the two. The suggested precursor–product relationship between lipotropin or B-endorphin (its 61–91 fragment) and the smaller pentapeptide fragments (enkephalins) is more tenuous because the larger forms are prominent in the pituitary gland and the pentapeptides are found primarily in the brain and gastrointestinal tract (8). Vasoactive intestinal polypeptide has been found in the gastrointestinal tract and in the central nervous system but fragments of this peptide have not been observed in either location (10).

The finding of peptides resembling CCK and CCK-8 in the central nervous system raises intriguing questions about their physiologic function particularly with respect to their potential roles as satiety factors. The observation of Gibbs *et al.* (11, 12) that injection of purified CCK or CCK-8 evoked satiety, although pentagastrin and secretin did not, has suggested a negative feedback mechanism from the gastrointestinal tract as the causative mechanism. The concept that the CCK peptides that appear to be endogenous in the brain may be neuroregulators is an intriguing one. In an accompanying paper, we report on the cellular localization in rabbit brain of a peptide with immunologic specificity like that of the CCK-8 (13).

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