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Immunochemical Evidence for a Gastrin-Like Peptide in Insect Neuroendocrine System

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Evidence demonstrating that the neuroendocrine system (brain, corpus cardiacum, and corpus allatum) of the tobacco hornworm, *Manduca sexta* (L.), contains a peptide that is gastrin-like in its antigenicity, size, and susceptibility to degradative enzymes is presented. No immunoreactive gastrin was found in gut extracts from four species of insects. These results suggest the existence of a peptide in insect nervous tissue having structural similarities to vertebrate gastrin.

There is much interest in the origin and evolution of polypeptides in vertebrates that may be common to both nerve cells of the brain and gastrointestinal endocrine-like cells (Pearse, 1976). These peptides include gastrin (Vanderhaegen *et al.*, 1975), somatostatin (Brazeau *et al.*, 1973; Arimura *et al.*, 1975), vasoactive intestinal peptide (Said and Rosenburg, 1976), and substance P (Hokfelt *et al.*, 1975; Pearse and Polak, 1975). For a more precise picture of the molecular evolution of these materials, a search for their presence in the invertebrate phyla is essential. Straus *et al.* (1975) have recently demonstrated the presence of gastrin-like immunoreactive peptides in the blood and gastrointestinal tissues from two molluscan species. We have conducted a survey for immunoreactive gastrin in insect tissues and report here the identification of a gastrin-like polypeptide in the neuroendocrine system (brain, corpus cardiacum, and corpus allatum) of the tobacco hornworm, *Manduca sexta* (L.). No gastrin was found in gut tissues.

MATERIALS AND METHODS

Insects. The test insects were obtained as follows: Eggs of *Manduca sexta* were a gift from Dr. J. Reinecke (Agricultural Research Service, U.S. Department of Agriculture, Fargo, North Dakota), and larvae were reared at 28° and 60% relative humidity with a 16-hr light–8-hr dark photoperiod and a standard diet (Bell and Joachim, 1976). *Dermestes maculatus* De Geer larvae were from the U.S. Grain Marketing Research Center Laboratory. Larvae of *Musca domestica* (Linn.) and *Musca autumnalis* De Geer were from Dr. C. Pitts (Department of Entomology, Kansas State University, Manhattan, Kansas).

Dissections and tissue extractions. Adults and larvae were anesthetized by cooling to 4° before dissection of midguts, brain, or complexes of brain, corpus cardiacum, and corpus allatum by standard techniques (Schneiderman, 1967). Tissue specimens were rinsed in saline solution containing 1 mg ml^{-1} of bovine serum albumin[1] (Sigma), 1 m*M* diisopropylphosphorofluoridate, and 5000 units ml^{-1} of the protease inhibitor Trasylol (FBA Pharmaceuticals, New York, New York). Tissues were homogenized by using small glass tissue grinders in 0.02 *M* ammonium bicarbonate, pH 8.6, containing 1.0 mg ml^{-1} of albumin and the proteinase inhibitors, and were extracted in 10 vol of boiling water (0.1 g ml^{-1}). The heat-treated homogenate was clarified by centrifugation at 10,000*g* for 10 min at 4°, freeze-dried, and stored at −20°. For blood analysis, hemolymph was heated at 70° for 30 min. Serum was collected after centrifugation and concentrated by ultrafiltration with a UM-05 filter (Amicon).

Column chromatography and radioimmunoassays. The neuroendocrine tissue extract [1 ml, 320 pg

[1] Mention of a proprietary product does not constitute an endorsement by the U.S. Department of Agriculture.

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equivalents of human gastrin-I (Yalow and Berson, 1970)] was gel filtered at 24° on a column (0.9 × 140 cm) of Bio-Gel P-10 (Bio-Rad Laboratories) by using 0.02 *M* ammonium bicarbonate, pH 8.6, and 1.0 mg ml^{-1} of albumin. One-milliliter fractions were collected and freeze-dried to remove solvent and salt; the residue was subjected to gastrin radioimmunoassay using the standard kit from Schwarz/Mann. The gastrin-I antibody employed has high association constants for sulfated and nonsulfated forms of human gastrin (Gregory *et al.*, 1964). Hemolymph and midgut tissue extracts were assayed similarly or diluted 1:10 or 1:25 for assay. All values reported were obtained by evaluation of experimental data against standard curves prepared with the heptadecapeptide form of human gastrin-I. The limit of detection of human gastrin is 25 pg with this procedure.

RESULTS AND DISCUSSION

Although insects generally lack an acid digestive process analogous to the gastric action in the stomach of vertebrates, it has been reported that certain insects have regions of quite acid pH in the midgut (Fraser *et al.*, 1961; Sinha, 1975). These reports suggested to us that insects, like vertebrates, may have a gastrin-like polypeptide that stimulates the secretion of gastric acid. This hypothesis led us to search for such a peptide in the insect digestive system by using a radioimmunoassay for human gastrin. As shown in Table 1, no gastrin-like immunoreactive components were found in gut tissues of two dipterans, *Musca domestica* (housefly) and *M. autumnalis* (face fly); a coleopteran, *Dermestes maculatus* (hide beetle); and a lepidopteran, *Manduca sexta* (tobacco hornworm). For control experiments, pooled blood and pooled nervous tissue samples (brain, corpus cardiacum, and corpus allatum) from the hornworm were also assayed. No immunoreactive gastrin was detected in hemolymph, but high levels were evident in the neuroendocrine system (from 0.8 to 1.8 pg equivalents of human gastrin per complex).

In order to determine whether the component(s) in the neuroendocrine system were physically similar to vertebrate gastrin, we subjected the *Manduca* extract to gel filtration on Bio-Gel P-10 (Fig. 1). Immunoreactive gastrin was detected as a single peak eluting at fraction 45. The elution volume of this component was slightly smaller than that of human iodinated gastrin [fraction 47 (Gregory *et al.*, 1964)], suggesting a similar or perhaps slightly larger molecular weight for the insect peptide ($MW_{app} = 2.3 \times 10^3$). The amount of immunoreactive peptide present in fractions 42–46 of Fig. 1 corresponded to approximately 12 ng equivalents of human gastrin per gram of insect nervous tissue. This figure should be regarded as a lower

TABLE 1
GASTRIN-LIKE IMMUNOREACTIVITY IN INSECT TISSUES

Insect	Stage at dissection[a]	Tissue[b]	Human gastrin equivalents (pg)[c]
Musca domestica	L	Whole body (100)	<25
	L	Midgut (100)	<25
Musca autumnalis	L	Midgut (100)	<25
Dermestes maculatus	L	Midgut (100)	<25
Manduca sexta	L	Midgut (1)	<25
	L	Hemolymph (50)	<25
	A	Brain (100)	105 ± 25
	A	Brain–corpus cardiacum–corpus allatum (100)	140 ± 35

[a] L, larval; A, adult.
[b] Number of insects, tissues, or milliliter equivalents pooled, extracted, and assayed given in parentheses.
[c] Mean values ± standard error.

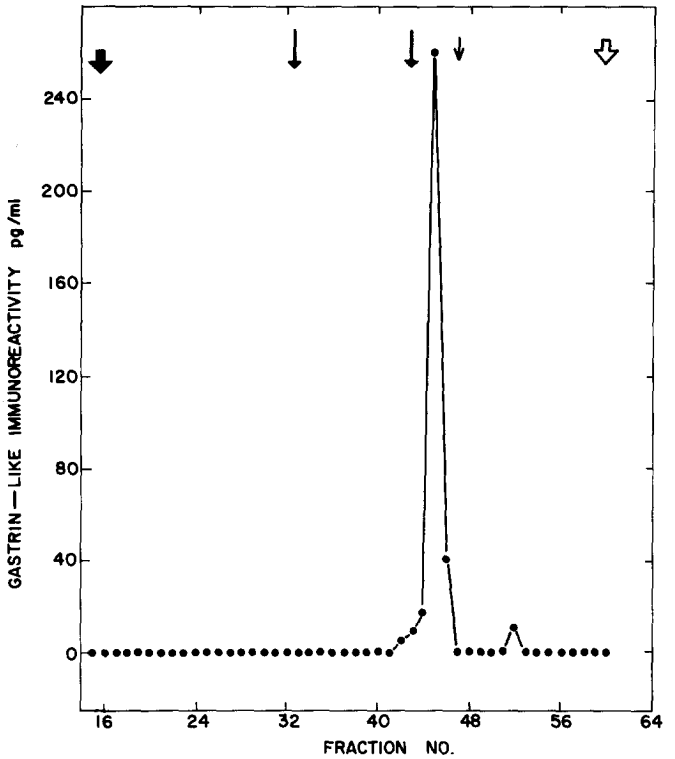

FIG. 1. Profile of gastrin-like immunoreactivity obtained after gel filtration of an extract of 270 neuroendocrine complexes from adult *Manduca sexta*. The elution positions of standards from the same column are indicated by the vertical arrows which represent, from left to right, bovine serum albumin (void volume), insulin, glucagon, ^{125}I-labeled human gastrin, and [^{14}C]leucine.

limit since affinity of the antibody for the insect material may well be less than that for human gastrin. The value is lower than the amount of immunoreactivity found in vertebrate nervous systems [200–400 ng (Vanderhaegen *et al.*, 1975)], but it is similar to that found for cholecystokinin (CCK)-like immunoreactivity in vertebrate brain tissue [20–50 ng/g (Dockray, 1976)]. There is structural homology between gastrin and CCK (Dockray, 1977); CCK cross-reacts in varying degrees with nearly all gastrin antisera and it is possible that the peptide found in insect tissue resembles CCK more closely than gastrin.

The insect gastrin-like peptide (200 pg) maintained its integrity on refractionation and exhibited no change in specific immunoreactivity when incubated for 20 min at 37° with trypsin (Sigma) at a concentration of 1 mg ml^{-1}. However, immunoreactivity disappeared after incubation with Pronase (Calbiochem) under similar conditions. This stability of insect gastrin is in accordance with that of vertebrate gastrin, which does not contain a basic amino acid residue, at least in those regions of the molecule that elicit antigenicity (Gregory *et al.*, 1964).

The function of the gastrin-like peptide in insects is not known. Since the neuroendocrine glands are not directly associated with the digestive system and also, since no gastrin was found in the circulatory system, it is unlikely that the polypeptide plays a role in digestion. The neural origin of insect gastrin might suggest a neurotransmitter function. The insect neurosecretory system is quite similar in terms of polypeptide components to the vertebrate pancreas

(Ganong, 1971; Tager *et al.*, 1976; Braaten *et al.*, 1976). The presence of glucagon-like, insulin-like, and gastrin-like polypeptides in both insect and vertebrate tissues indicates that all three peptide families have evolutionary histories dating back to the ancestor common to insects and vertebrates and is consistent with the hypothesis that these polypeptides are derived from neuroectodermal cells (Fox and Fox, 1974; Pearse, 1976).

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