

Glucagon-like immunoreactivity in insect corpus cardiacum

SINCE Steele attributed hyperglycaemic activity to the corpus cardiacum of the cockroach neurosecretory system¹⁻³, it has been demonstrated in other species of Orthoptera⁴⁻¹⁰ and in Diptera^{11,12} and Hymenoptera¹³. Although extracts of corpora cardiaca from the blowflies *Phormia*¹¹ and *Calliphora*¹⁴ and the silk moth *Cecropia*¹⁵ seem to be inactive, the hyperglycaemic hormone is liberated from the *Calliphora* gland by electrical¹² or mechanical¹⁴ stimulation *in situ*. Similarly, extracts from *Locusta*^{6,16} and *Phormia*¹¹ are poorly active intraspecifically, but are hyperglycaemic when injected into the cockroach. The nutritional state of the recipient is apparently crucial in determining the extent of the response¹¹.

In the best studied insects, the hyperglycaemic hormone functions similarly to glucagon in vertebrates: the hormone increases carbohydrate levels in the circulating haemolymph

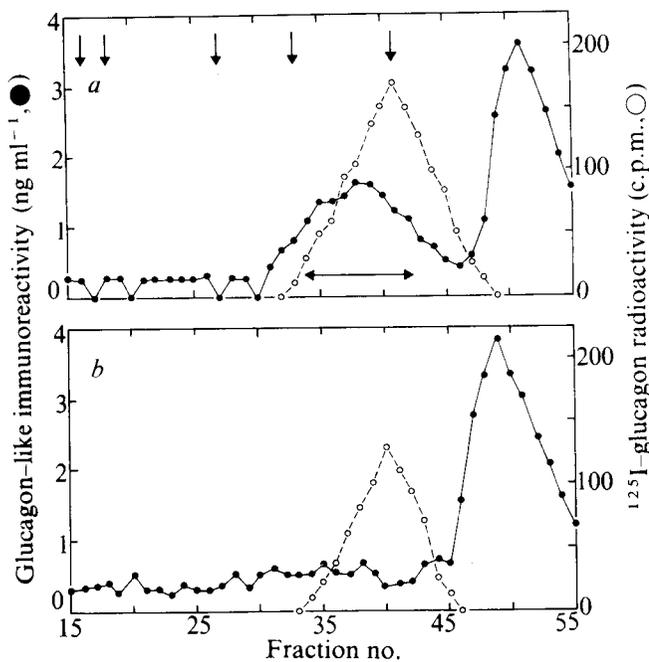


Fig. 1 Gel filtration of extracts of corpus cardiacum/corpus allatum complexes and brains from adult *M. sexta*. Tissue (21 complexes or brains) was suspended in 0.1 M Tris, 0.05 M NaCl, 0.25% bovine serum albumin, 1 mM diisopropylphosphorofluoridate (DFP), pH 7.6, lyophilised and extracted with 0.3 ml of 7 M guanidine hydrochloride containing a trace amount of ¹²⁵I-glucagon. The extracts were separately applied to a column (0.9 × 42 cm) of BioGel P-10 equilibrated at 4 °C with Tris-albumin buffer containing pancreatic kallikrein inhibitor (300 U ml⁻¹) instead of DFP, and the components eluted at a flow rate of 5 ml h⁻¹. The 0.6 ml fractions were counted for radioactivity and 0.15 ml aliquots of each were removed for radioimmunoassay essentially as described²⁵. The antiglucagon serum was a gift from Dr L. Heding, Novo Laboratories, Copenhagen. Glucagon-like immunoreactivity (left axis, solid lines) and ¹²⁵I-glucagon radioactivity (right axis, dashed lines) were recorded for the complexes (a) and the brains (b). The elution positions of protein standards from the same column are indicated by the vertical arrows (a) which represent from left to right, albumin (void volume), soybean trypsin inhibitor (STI), cytochrome c, pancreatic trypsin inhibitor, and glucagon or ¹²⁵I-glucagon, respectively. Fractions 34 to 42 indicated by the horizontal arrow (a) were pooled for further analysis.

in vivo and stimulates glycogenolysis and the activation of phosphorylase in the fat body *in vitro*^{5,6,17,18}. By several criteria, the insect hormone also seems to be a low molecular weight polypeptide^{2,6,8,10,13,16}. We report here the identification

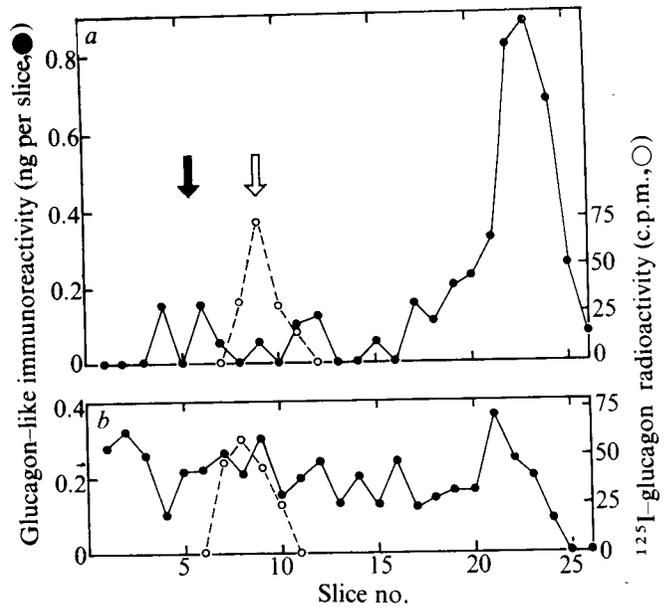


Fig. 2 Polyacrylamide gel electrophoresis at pH 8.7 of extracts of corpus cardiacum and corpus allatum from *M. sexta*. Tissue (9 gland pairs) was extracted with 0.05 ml of 8 M urea containing trace amounts of ¹²⁵I-glucagon and bromphenol blue. The extracts were applied to 10% polyacrylamide gels²⁸ containing 8 M urea (0.4 × 6 cm) and were subjected to electrophoresis at 22 °C. Gels were sliced into 1.5-mm sections and placed in 0.5 ml of the buffer used for chromatography before counting for radioactivity. After elution of the peptide components during 24 h at 4 °C, the supernatant fluid was withdrawn and subjected to immunoassay. Glucagon-like immunoreactivity (left axis, solid lines) and ¹²⁵I-glucagon radioactivity (right axis, dashed lines) were recorded for the corpus cardiacum (a) and the corpus allatum (b) extracts. Migration was from cathode (left) to anode. The position of the tracking dye is coincident with the right axis. The solid and open arrows (a) indicate the migration position of porcine glucagon and ¹²⁵I-glucagon respectively. The additional negative charge in iodinated glucagon at pH 8.7 increases its anodal mobility relative to that of the unlabelled hormone. Control experiments showed that the presence of urea or other gel components did not alter the sensitivity of the immunoassay.

of a highly acidic peptide with glucagon-like immunoreactivity from corpora cardiaca of the adult tobacco hornworm *Manduca sexta*.

A survey for immunoreactive glucagon in tissues of *M. sexta* showed that reactive peptides were present in corpus cardiacum/corpus allatum complexes and haemolymph, but not in brain, aorta, recurrent nerve or fat body. The component from the complexes was eluted slightly ahead of ¹²⁵I-glucagon (molecular weight 3,500) during gel filtration on BioGel P-10 (Fig. 1a). The elution volume of the immunoreactive component corresponded to that of a 4,500-dalton peptide. As Fig. 1b shows, gel filtration of an extract of brains from *M. sexta* yielded no similar peak of glucagon-like immunoreactivity. The peak of apparent reactivity at the far right of each profile in Fig. 1 is caused by interference in the assay by guanidine hydrochloride and represents the inclusion volume of the column.

A closer examination of the distribution of the immunoreactive peptide showed that it was primarily associated with the corpus cardiacum rather than with the corpus allatum (Fig. 2a, slices 21-25). Its rate of migration during electrophoresis at pH 8.7 is in agreement with its low molecular weight as determined by gel filtration, and further indicates a high acidity. Nevertheless, the abilities of the serially diluted, pooled fractions 34-42 (Fig. 1a) and porcine glucagon to compete with ¹²⁵I-glucagon for binding to the antibody

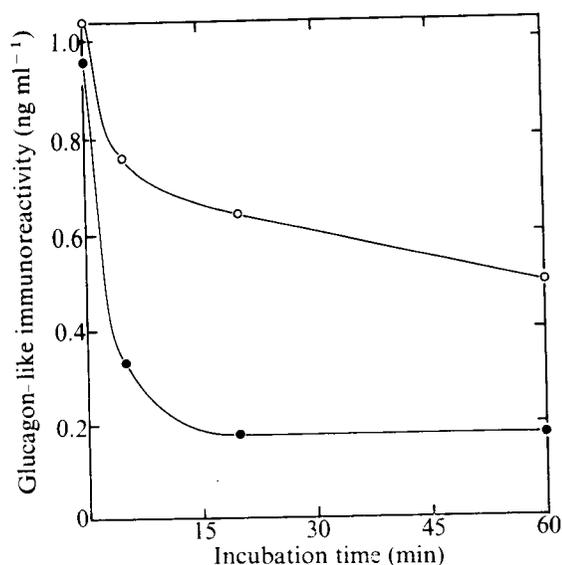


Fig. 3 Effect of trypsin on the glucagon-like immunoreactivity in a gel-filtered extract of corpus cardiacum/corpus allatum complexes from *M. sexta*. Samples (0.4 ml) of the pooled fractions 34–42 from Fig. 1 or of a solution of porcine glucagon (1 ng ml⁻¹ in chromatography buffer) were incubated with diphenylcarbamyl chloride-treated trypsin (0.075 mg) at 25 °C for the indicated intervals. The reaction was stopped by the addition of 0.1 ml of a solution of STI (8 mg ml⁻¹). All samples were subjected to the immunoassay and glucagon-like immunoreactivity was recorded for *M. sexta* (○) and glucagon (●). The data points at zero time were obtained by adding STI to the appropriate solution before the addition of trypsin. Control experiments showed that the presence of trypsin plus STI did not alter the sensitivity of the assay.

decreased in parallel. Although the immunoreactivity profile of Fig. 2b suggests that corpus cardiacum contains a single, major glucagon-like peptide, two or more rapidly migrating forms might not be separated under the conditions used here.

Since mammalian glucagon¹⁹ and the insect hyperglycaemic hormone^{5,13} are sensitive to proteolytic degradation, we compared the susceptibilities of the immunoreactive component and porcine glucagon to trypsin (Fig. 3). Although the immunoreactivities of both the insect peptide and glucagon are diminished by digestion with trypsin, the former is degraded either more slowly or to a lesser extent. This result may be a consequence of the higher acidity of the insect peptide (Fig. 2a). Quantitation of glucagon-like immunoreactivity after gel filtration or after polyacrylamide gel electrophoresis indicated that each pair of corpora cardiaca from *M. sexta* contains 0.4–0.5 ng equivalent of glucagon. This value should be regarded as a lower limit, however, since affinity of our antibody for the insect material may well be less than for porcine glucagon.

Examination of sequence data shows that the structure of the vertebrate hyperglycaemic hormone glucagon has been well conserved during evolution: the human,²⁰ bovine,²¹ and porcine¹⁹ hormones are identical, although the structures of the corresponding, slowly evolving cytochromes *c* differ by an average of 7% (ref. 22). Similarly, avian glucagon differs from the human hormone by 7% (ref. 23), whereas the difference between the corresponding cytochromes is about 12% (ref. 22). Assuming from these limited data that the rate of mutational

acceptance for glucagon is only about half that for cytochrome *c* or 1.5 accepted point mutations per 100 residues per 100 Myr,²⁴ we expect that a glucagon-like peptide might have changed by only 15% during the approximately 900 Myr since the divergence of insects and mammals²⁴. Neither this estimated extent of change nor the apparently higher molecular weight of the insect peptide would be likely to preclude reactivity with antibodies directed toward mammalian glucagon²⁵.

Weins and Gilbert first noted the nearly identical physiological actions of the insect hyperglycaemic hormone and vertebrate glucagon⁵. The tissue distribution of the glucagon-like immunoreactivity in *M. sexta* parallels the distribution of the hyperglycaemic hormone in other insects^{1,18,26,27}. Furthermore, both immunoreactivity and hyperglycaemic activity are sensitive to tryptic degradation and behave similarly during gel filtration at neutral pH¹³. The high acidity of the immunoreactive peptide is also consistent with the finding that hormone activity does not adsorb to cation-exchange resins^{10,13}. Although these chemical and physical similarities suggest that the insect hyperglycaemic hormone and the glucagon-like peptide are the same, final proof of their identity must await purification and sequence determination of the hyperglycaemic peptide.

We thank Dr. R. A. Bell for *M. sexta* eggs and L. Hendricks for rearing the insects. H.S.T. acknowledges financial support from the Sprague Memorial Fund of the University of Chicago, the Kroc Foundation and the National Institute of Arthritis, Metabolic and Digestive Diseases.

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Received February 11; revised March 7, 1975.

- 1 Steele, J. E., *Nature*, **192**, 680–681 (1961).
- 2 Steele, J. E., *Gen. comp. Endocr.*, **3**, 46–52 (1963).
- 3 Goldsworthy, G. J., and Mordue, W., *J. Endocr.*, **60**, 529–558 (1974).
- 4 Bowers, W. S., and Friedman, S., *Nature*, **198**, 685 (1963).
- 5 Wiens, A. W., and Gilbert, L. I., *J. Insect Physiol.*, **13**, 779–794 (1967).
- 6 Goldsworthy, G. J., *J. Insect Physiol.*, **15**, 2131–2140 (1969).
- 7 Keeley, L. L., and Friedman, S., *Gen. comp. Endocr.*, **8**, 129–134 (1967).
- 8 Brown, B. E., *Gen. comp. Endocr.*, **5**, 387–401 (1965).
- 9 Dutrieu, J., and Gourdoux, L., *C. r. hebd. Séanc. Acad. Sci.*, **265**, 1067–1070 (1967).
- 10 Natalizi, G. M., Pansa, M. C., D'Ajello, V., Casaglia, O., Bettini, S., and Frontali, N., *J. Insect Physiol.*, **16**, 1827–1836 (1970).
- 11 Friedman, S., *J. Insect Physiol.*, **13**, 397–405 (1967).
- 12 Normann, T. C., and Duve, H., *Gen. comp. Endocr.*, **12**, 449–459 (1969).
- 13 Natalizi, G. M., and Frontali, N., *J. Insect Physiol.*, **12**, 1279–1287 (1966).
- 14 Natalizi, G. M., and Norman, T. C., *J. Insect Physiol.*, **20**, 1189–1192 (1974).
- 15 Vejbjerg, K., and Gilbert, L. I., *Comp. Biochem. Physiol.*, **21**, 145–159 (1967).
- 16 Mordue, W., and Goldsworthy, G. J., *Gen. comp. Endocr.*, **12**, 360–369 (1969).
- 17 Goldsworthy, G. J., *J. Insect Physiol.*, **15**, 2131–2140 (1969).
- 18 Goldsworthy, G. J., *Gen. comp. Endocr.*, **14**, 78–85 (1970).
- 19 Bromer, W. W., Sinn, L. G., and Behrens, O. K., *J. Am. chem. Soc.*, **79**, 2807–2810 (1957).
- 20 Thomsen, J., Kristiansen, K., Brunfeldt, K., and Sundby, F., *FEBS Lett.*, **21**, 315–319 (1972).
- 21 Bromer, W. W., Boncher, M. E., and Koffenberger, J. E., Jr., *J. Biol. Chem.*, **246**, 2822–2827 (1971).
- 22 Dayhoff, M. O. (ed.), in *Atlas of protein sequence and structure*, **5**, D–8 (National Biomedical Research Foundation, Silver Spring, Maryland, 1972).
- 23 Sundby, F., Frandsen, E. K., Thomsen, J., Kristiansen, K., and Brunfeldt, K., *FEBS Lett.*, **26**, 289–293 (1972).
- 24 McLaughlin, P. J., and Dayhoff, M. O., in *Atlas of protein sequence and structure*, **5** (edit. by Dayhoff, M. O.), 50 (National Biomedical Research Foundation, Silver Spring, Maryland, 1972).
- 25 Tager, H. S., and Steiner, D. F., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2321–2325 (1973).
- 26 Ralph, C. L., and McCarthy, R., *Nature*, **203**, 1195–1196 (1964).
- 27 Highnam, K. C., and Goldsworthy, G. J., *Gen. comp. Endocr.*, **18**, 83–88 (1972).
- 28 Ornstein, L., and Davis, J. B., *Disc. electrophoresis* (Distillation Products Industries, Rochester, New York, 1962).