A neuropeptide of the blowfly *Calliphora vomitoria* with an amino acid composition homologous with vertebrate pancreatic polypeptide

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A neuropeptide purified from the brain of the blowfly (*Calliphora vomitoria*) that cross-reacts in a bovine pancreatic polypeptide radioimmunoassay has been subjected to amino acid analysis. The amino acid composition of the peptide shows homology with vertebrate pancreatic polypeptide species. Amounts of the neuropeptide calculated from amino acid analysis accord with those measured by the pancreatic polypeptide radioimmunoassay. These results suggest that the primary structure of the *Calliphora* neuropeptide is very similar to that of mammalian pancreatic polypeptides.

Biologically active peptides of the gastroenteropancreatic series of vertebrates have been shown by immunocytochemistry to be present in the nervous tissues of various invertebrates (Sundler *et al.*, 1977; Doerr-Schott *et al.*, 1978; Duve & Thorpe, 1979, 1980, 1981; Grimmelikhuijzen *et al.*, 1980; El-Salhy *et al.*, 1980; Yui *et al.*, 1980). Partial purification and characterization of some of these peptides from the blowfly *Calliphora vomitoria* [insulin (Duve *et al.*, 1979), gastrin (Dockray *et al.*, 1981) and pancreatic polypeptide (Duve *et al.*, 1981)], the tobacco hornworm *Manduca sexta* [insulin and glucagon (Tager *et al.*, 1976)] and the fruit fly *Drosophila melanogaster* [insulin (Le Roith *et al.*, 1981)] indicate that they resemble closely the mammalian counterparts physiocochemically, immunologically and biologically. We report here the purification and amino acid composition of *Calliphora* pancreatic polypeptide and show the chemical and immunological homology between molecules of this type from invertebrate and vertebrate sources.

Experimental and results

An acid/ethanol extract of 10⁶ heads of *Calliphora* (5 kg) was submitted to Sephadex-gel filtration (column dimensions, 14 cm × 100 cm; eluent 1 M acetic acid/0.05 M-NaCl). The fractions rich in

Abbreviation used: h.p.l.c., high-pressure liquid chromatography.

pancreatic polypeptide and insulin immunoreactivities (K_{av} 0.56) contained approx. 960 mg of protein, with 60 ng of both peptides present/mg of protein, as judged by radioimmunoassay (Duve *et al.*, 1981; see the legend to Fig. 1). After freeze-drying, half of this material was further purified on DEAE-cellulose as described in Fig. 1, where the insulin immunoreactivity was separated from the pancreatic polypeptide immunoreactivity. The fractions containing pancreatic polypeptide immunoreactivity were pooled and purified by h.p.l.c. with the successive use of Ultrasphere ODS (octadecylsilane) and Cyano (cyanopropyl) columns (Altex Scientific Inc., Berkeley, CA, U.S.A.) (Figs. 2a and 2b). Fraction 165 (Fig. 2b) was re-applied to an Ultrasphere ODS column (Fig. 2c). Fractions were immunoassayed for pancreatic polypeptide immunoreactivity and the peak fraction (fraction 10) was used for the amino acid analysis, which was carried out in duplicate (Table 1). The homology of the peptide analysed with vertebrate pancreatic polypeptides is suggested by the following characteristics. (i) The total number of amino acids present is 36 (based on the assumption that the molecular weight is approximately that of bovine pancreatic polypeptide (i.e. 4200). (ii) The amino acids present are, with minor variation, in proportions similar to the known vertebrate species. (iii) There is a lack of phenylalanine, histidine and lysine residues. (iv) The material was eluted in the expected position of pancreatic polypeptide on h.p.l.c. (Fig. 2c). Several factors indicate that the conformation

Fig. 1. DE-52 DEAE-cellulose chromatography of reconstituted freeze-dried material (462mg) from *Calliphora* brain

Column size, 1.5 cm $\times$ 30 cm; gradient, 20–400 mM ammonium acetate, pH 8.5, in the presence of 20% acetonitrile; flow rate, 20 ml/h; fractions, 4 ml. Samples (10 μ l) were immunoassayed for insulin (Duve *et al.*, 1979) and pancreatic polypeptide [homogeneous bovine pancreatic polypeptide assay, antibody to bovine pancreatic polypeptide; separation of bound and free form was by activated charcoal (Duve *et al.*, 1981)]. Yield of immunoreactive pancreatic polypeptide (PP), 15 μ g (50% recovery). A similar yield for insulin was also obtained (stippled peak). The histograms refer to immunoreactivity and the continuous line to A_{280} .

of the antigenic sites of the invertebrate peptide is the same as the mammalian species: (i) immunoassay dilution curves show linearity and parallelism with bovine pancreatic polypeptide standards; (ii) the amount of material recovered from fraction 10 (Fig. 2c) was found to be approx. 650 ng by the calculation from amino acid analysis and 670 ng in the bovine pancreatic polypeptide immunoassay. We conclude from these criteria that the primary structure of *Calliphora* pancreatic polypeptide is very similar to the known pancreatic polypeptide molecules of vertebrates and perhaps more especially those of mammals.

Discussion

There is considerable interest in the series of biologically active peptides of mammals and other vertebrates that occur in both brain and gut (Pearse, 1976; Hökfelt *et al.*, 1980). Immunocytochemical and biochemical studies have, however, outpaced physiological studies and, whereas the localization within tissues has been accurately mapped and the chemical characterization has been determined, the role of peptides such as cholecystokinin (Gibbs, *et al.*, 1973), vasoactive intestinal peptide (Fahrenkrug, 1979), insulin (Havrankova *et al.*, 1979) and

Fig. 2. H.p.l.c. of pancreatic polypeptide-immunoreactive peak

(a) shows preparative h.p.l.c. of the pancreatic polypeptide-immunoreactive peak from DEAE-cellulose chromatography. Column size, 1 cm $\times$ 15 cm; column material, Ultrasphere ODS; gradient, 40–80% methanol/1% trifluoroacetic acid; flow rate, 3 ml/min; 1 ml fractions. A_{280} , 0.32 absorbance unit full scale (AUFS). Samples (5 μ l) were immunoassayed for pancreatic polypeptide; 12 μ g of pancreatic polypeptide-immunoreactive material (PPI) was recovered (see the histogram). (b) shows analytical h.p.l.c. of 2 μ g of pancreatic polypeptide-immunoreactive peak from (a). Column size 0.4 cm $\times$ 15 cm; column material, Ultrasphere Cyano; gradient 20–60% acetonitrile/1% trifluoroacetic acid; flow rate, 1 ml/min; 200 μ l fractions. A_{280} , 0.04 absorbance unit full scale. Samples (5 μ l) were assayed for pancreatic polypeptide. Recovery 100%. Fraction no. 165 (1.2 μ g) was used for further purification. (c) shows analytical h.p.l.c. of fraction 165 (1.2 μ g of pancreatic polypeptide-immunoreactivity) from (b). Column size 0.4 cm $\times$ 25 cm. Column material, Ultrasphere ODS; gradient, 40–80% methanol/1% trifluoroacetic acid. Fraction 10 (670 ng) was used for amino acid analysis. The arrow indicates the expected elution position of bovine pancreatic polypeptide. A_{280} , 0.02 absorbance unit full scale. The histograms refer to immunoreactivity and the continuous lines to A_{280} in each Figure. PP, pancreatic polypeptide.

Table 1. *Amino acid composition of Calliphora neuropeptide and vertebrate pancreatic polypeptides*

Purified *Calliphora* neuropeptide was analysed by means of an automatic ion-exchange analyser (Joel) with both ninhydrin and *o*-phthaldialdehyde monitoring channels. Two separate analyses were obtained from 24 h acid hydrolysates of approx. 100 pmol each. Values for serine and threonine have been corrected for an estimated 30% loss during hydrolysis. Cysteine and tryptophan were not determined. Results from the two analyses are given (ninhydrin detection) with the nearest integers in parentheses. Integer values were normalized to four residues of tyrosine. Values for vertebrate pancreatic polypeptides are from Floyd *et al.* (1977).

	Content (residues/mol)							
	<i>Calliphora</i>			Bovine	Porcine	Ovine	Human	Avian
Asx	3.4	3.1	(3)	3	3	3	4	6
Thr	2.4	2.5	(2)	2	2	2	2	2
Ser	1.3	1.2	(1)	0	0	1	0	1
Glx	5.7	6.1	(6)	6	5	6	4	4
Pro	3.8	4.2	(4)	5	5	4	5	4
Gly	1.9	1.9	(2)	1	1	1	1	3
Ala	3.9	3.7	(4)	5	5	5	5	1
Cys	—	—	—	0	0	0	0	0
Val	1.2	1.4	(1)	0	1	0	1	3
Met	1.4	1.6	(1)	2	2	2	2	0
Ile	2.2	1.6	(2)	1	1	1	1	1
Leu	2.5	2.8	(3)	3	3	3	3	3
Tyr	4.0	4.0	(4)	4	4	4	4	4
Phe	0	0	(0)	0	0	0	0	0
His	0	0	(0)	0	0	0	0	1
Lys	0	0	(0)	0	0	0	0	0
Arg	3.1	3.3	(3)	4	4	4	4	3
Trp	—	—	—	0	0	0	0	0

pancreatic polypeptide (Loren *et al.*, 1979) within the central nervous system is equivocal.

Evidence is now accumulating to suggest that these so-called brain/gut bioactive peptides of the vertebrates are also present in a wide variety of invertebrates, especially within the nervous tissue. The evidence comes from: (i) immunocytochemical studies using antibodies directed against mammalian peptides; (ii) detection in tissue extracts by radioimmunoassay using mammalian standards; (iii) bioassay of partially purified peptides (Tager *et al.*, 1976; Duve *et al.*, 1979; Le Roith *et al.*, 1981). Taken together, these studies only suggest that there is some degree of identity with the mammalian peptides (cf. Julliard *et al.*, 1980). Our own immunochemical studies on the brain of *Calliphora* with bovine pancreatic polypeptide antibodies have revealed a series of strongly immunoreactive neurons in well defined regions of the brain (Duve & Thorpe, 1980) and, furthermore, pancreatic polypeptide immunoreactive material is readily identifiable in extracts (Duve *et al.*, 1981). The results presented here demonstrate that for at least one gastroenteropancreatic peptide in one invertebrate, the cross-reactivity observed in immunocytochemistry and radioimmunoassay is not due to a corresponding amino acid sequence occurring by chance in an

alien peptide but rather that it is derived from molecular homology.

The conclusion from the present study is that the peptide previously isolated from a few mammalian and avian species and referred to as pancreatic polypeptide has probably existed throughout a much longer period of evolution than previously supposed. The fact that it is present in the brain of an insect is further evidence that peptides of the gastroenteropancreatic series were perhaps originally neurotransmitters, neurohormones or neuromodulators that secondarily adapted to a gastroenteropancreatic function. A revision of the nomenclature for certain of these peptides may have to be considered in the future.

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