However, it is possible that the cultured chondrocytes themselves, in response to GH *in vitro*, produce somatomedins or other growth factors that may be of importance for the effect of the hormone. Interestingly, it has been shown that the stimulatory effect of hGH *in vitro* on the growth of cultured human fibroblasts is not expressed when the culture medium is changed frequently[29]. This suggests that fibroblasts indeed secrete 'factors' into the incubation medium that are important for the effect of GH on fibroblasts. The extent to which the effect of GH on chondrocyte growth is mediated or modulated by 'local somatomedins' remains to be shown. Nevertheless, our results demonstrate that GH has the ability to interact directly with chondrocytes from rabbit ear and rat rib growth cartilage, causing an increased DNA synthesis.

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Human preprovasoactive intestinal polypeptide contains a novel PHI-27-like peptide, PHM-27

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Vasoactive intestinal polypeptide (VIP), a 28-amino acid peptide originally isolated from porcine duodenum[1], is present not only in gastrointestinal tissues but also in neural tissues, possibly as a neurotransmitter, and exhibits a wide range of biological actions (for example, relaxation of smooth muscle, stimulation of intestinal water and electrolyte secretion and release of insulin, glucagon and several anterior pituitary hormones)[2–5]. As the structure of porcine and bovine VIP shows several similarities to those of mammalian glucagon, secretin and gastric inhibitory peptide (GIP), VIP is considered to be a member of the glucagon–secretin family[2]. Recently, we have found that VIP is synthesized from a precursor, pro-VIP (molecular weight (M_r) 17,500), in human neuroblastoma cells and that the primary translation product of the mRNA encoding VIP is prepro-VIP (M_r 20,000)[6]. In an attempt to elucidate the primary structure of the precursor, we have now cloned the DNA sequence complementary to the mRNA coding for human VIP and analysed the nucleotide sequence. The entire amino acid sequence of the precursor, deduced from the nucleotide sequence, indicates that the precursor protein contains not only VIP but also a novel peptide of 27 amino acids. The peptide, designated PHM-27, differs by only 2 amino acids from PHI-27, a peptide recently isolated from porcine intestine[7], and is also closely related in sequence to VIP.

Poly(A)-containing RNA was isolated from human neuroblastoma cells as described previously[6]. As the content of mRNA for the VIP precursor in the poly(A)-containing RNA was found to be only 0.12% by the cell-free translation assay using a rabbit reticulocyte lysate followed by immunoprecipitation with anti-VIP antiserum, the poly(A)-containing RNA was fractionated by sucrose gradient centrifugation[8,9]. Double-stranded cDNA was synthesized using the partially purified mRNA as a template and inserted into the *Pst*I endonuclease cleavage site of the plasmid pBR322 by the oligo(dG)-oligo(dC) tailing method. *Escherichia coli* χ1776 was transformed with the recombinant DNA. To identify the transformants containing the recombinant plasmid carrying the cDNA sequence for the VIP precursor, the plasmid DNAs were isolated from the transformants and analysed by a hybridization–translation assay. A plasmid DNA positive in the assay was designated as pVIP-1, and the cDNA in pVIP-1 was subjected to nucleotide sequence analysis. As the cDNA insert (nucleotide residues 53–1,279 in Fig. 1) did not contain the entire protein-coding sequence, we determined the sequence of the 5′-terminal region by the primer extension method. The 5′-^{32}P-labelled anticoding strand of the *Hgi*AI–*Hae*III fragment (nucleotide residues 53–78 in Fig. 1) that hybridized with the poly(A)-containing RNA from human neuroblastoma cells, was elongated by reverse transcriptase. The nucleotide sequence of the elongated DNA was also analysed.

Figure 1 shows the nucleotide sequence of the mRNA for the precursor protein. Nucleotide residues 53–1,279 were deduced from the nucleotide sequence of the cloned cDNA and nucleotides −30–52 were deduced from the nucleotide sequence of the elongated cDNA with the primer. The amino acid sequence (residues 125–152) deduced from the nucleotide residues 373–456 corresponds precisely to the amino acid sequence of porcine and bovine VIP[2]. Although the amino acid sequence of human VIP has not been determined, the amino acid composition of human VIP isolated from colon tissue is identical to those of porcine and bovine VIP[10]. Therefore, it is reasonable to assume that the amino acid sequence (residues 125–152) corresponds to that of human VIP. The amino-terminus of human VIP is preceded by a pair of basic amino acid residues (Lys-Arg) known to be frequent sites for the post-translational processing of hormone precursors[11]. A glycine residue and a pair of basic amino acid residues lie adjacent to the carboxy-terminal Asn residue. As with other polypeptide precursors[12–14], the glycine residue seems to serve as an amino donor to the carboxy-terminus of human VIP, because both porcine and bovine VIP contain a carboxy-terminal Asn-NH_2 residue[2]. The identity of porcine, bovine and human VIP suggests that VIP, like glucagon, may have been conserved during evolution in mammals.

The most interesting finding is that the deduced amino acid sequence at residues 81–107, which shows a remarkable similarity to that of VIP, is almost identical to that of porcine PHI-27

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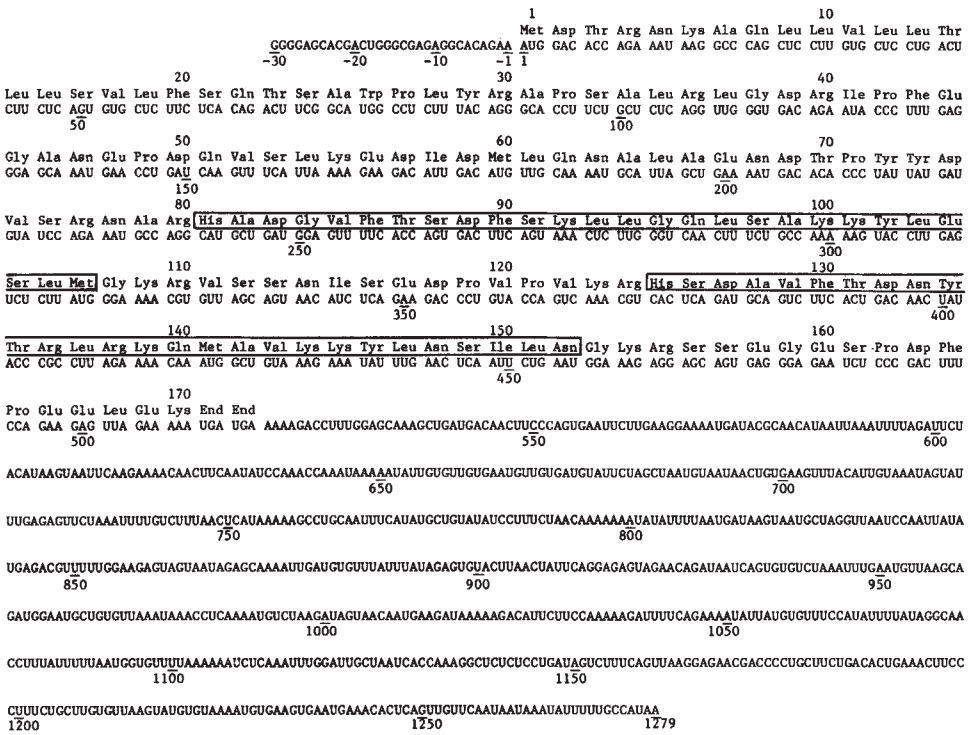

Fig. 1 Primary structure of human prepro-VIP/PHM-27 mRNA and protein. Nucleotide residues are numbered in the 5′ to 3′ direction, beginning with the first residue of the AUG triplet encoding the initiative methionine, and the nucleotides on the 5′ side of residue 1 are indicated by negative numbers. The deduced amino acid sequence is given above the nucleotide sequence, and amino acid residues are numbered beginning with the initiative methionine. The VIP and PHM-27 sequences are boxed. The two translation termination codons, UGA (nucleotides 511–516), were found at a site 55 nucleotides downstream from the carboxy-terminus of VIP. The translational initiation site was assigned to the methionine codon, AUG (nucleotides 1–3), that is, the first AUG triplet in the deduced mRNA sequence, because the sequence of ~20 amino acid residues starting with this methionine (residue 1) exhibits a feature characteristic of the signal peptide at the amino-terminal region of a secretory protein[11], and the molecular weight (19,169) of the precursor protein (170 amino acid residues) calculated by this assignment was consistent with that of the precursor protein, prepro-VIP (M_r 20,000), estimated by SDS-polyacrylamide gel electrophoresis[6]. It has been shown that the signal peptide generally contains a region rich in hydrophobic amino acid residues with large side chains and terminates in a residue having a small neutral side chain (alanine, serine or glycine)[11]. Therefore, a possible site for cleavage of the signal peptide in the precursor seems to exist after the serine residue at position 21 (or after the serine or alanine residue at positions 24 or 25). A possible glycosylation site, Asn-X-Thr[20], is located at amino acid residues 68–70, suggesting that the VIP/PHM-27 precursor protein may be a glycoprotein. The 3′-noncoding region of the mRNA is unusually long (>700 nucleotides excluding the poly(A) tract) and contains three copies of the sequence AAUAAA (nucleotide residues 644–649, 978–983 and 1,260–1,265) which precede the site of polyadenylation in many eukaryotic mRNAs[21].

Methods: Poly(A)-containing RNA, which was isolated from human neuroblastoma cells as described previously[6], was applied to a linear 15–30% sucrose gradient, and centrifuged at 60,000 r.p.m. at 20 °C for 6 h in a Beckman SW 60 Ti rotor[8,9]. The 12–13S fraction which contained the mRNA for the VIP precursor was ethanol-precipitated. The content of the mRNA for the precursor in the partially purified mRNA was found to be ~1.5%. Single-stranded cDNA was synthesized from the partially purified mRNA with avian myeloblastosis virus reverse transcriptase[22] (provided by Dr Y. Ikawa, Cancer Institute, Tokyo). Double-stranded cDNA was synthesized from single-stranded cDNA with a large fragment of *E. coli* DNA polymerase I (BRL)[22]. The double-stranded cDNA was treated with S_1 nuclease[23] (Sankyo) and dC-tailed with calf thymus terminal transferase[24] (P-L Biochemicals). The dC-tailed double-stranded cDNA was then annealed to dG-tailed *Pst*I-cut pBR322[24]. Transformation of *E. coli* χ1776 was carried out with the recombinant DNA[25]. About 1,500 tetracycline-resistant transformants were obtained and then screened by the colony hybridization method[26] with ^{32}P-labelled DNA complementary to the partially purified mRNA, yielding about 200 hybridization-positive clones. The plasmid DNA (about 2 μg) prepared from the hybridization-positive clone was immobilized to a dibenzyloxymethyl (DBM) paper[27] (1 cm^2). 3 μg of the poly(A)-containing RNA from human neuroblastoma cells with 30 μg of rat liver RNA and 10 μg of polyadenylic acid (P-L Biochemicals) were hybridized with the plasmid DNA immobilized on a DBM paper in 15 μl of hybridization buffer[27] (50% formamide, 0.6 M NaCl, 4 mM EDTA, 40 mM Tris-HCl, *p*H 7.8, 0.1% SDS) at 37 °C for 18 h. After hybridization, the paper was washed three times with 3 ml of the hybridization buffer at 37 °C for 1 h and washed once with 3 ml of 0.3 M NaCl containing 2 mM EDTA and 20 mM Tris-HCl, *p*H 7.8, at 25 °C for 30 min. The hybridized RNA was eluted from the washed paper with an elution buffer[27] (99% formamide, 10 mM Tris-HCl, *p*H 7.8) at 65 °C for 5 min and precipitated with 10 μg of wheat germ tRNA (BRL) using ethanol. The precipitate was washed twice with 1 ml of 70% ethanol, dissolved in 10 μl of H_2O and translated by the cell-free translation system of a rabbit reticulocyte lysate[6] with ^{35}S-methionine (1,500 Ci mmol^{-1}; Amersham). The translation product was immunoprecipitated with antiserum to synthetic porcine VIP[28] and analysed by SDS-polyacrylamide gel electrophoresis, followed by autoradiofluorography[6]. A plasmid DNA positive in the hybridization–translation assay was designated as pVIP-1. The cDNA insert in pVIP-1 was fragmented by restriction endonucleases. DNA sequence analysis on both strands was performed by the method of Maxam and Gilbert[29]. For synthesis of DNA complementary to the 5′-terminal region of the mRNA for the VIP precursor, the *Hgi*AI–*Hae*III fragment (nucleotide residues 53–78) of the cDNA insert was denatured and applied to an 8% polyacrylamide sequence gel in the presence of 8.3 M urea[29] for strand separation. The 5′ end of the 26-nucleotide long anticoding strand of the fragment was dephosphorylated with alkaline phosphatase (P-L Biochemicals) and labelled with T4 polynucleotide kinase (Takara Shuzo, Kyoto, Japan) and 5′-[γ-^{32}P]ATP (3,000 Ci mmol^{-1}; Amersham)[29]. The primer extension reaction was carried out according to the method of Nagata *et al.*[30]. The ^{32}P-labelled anticoding strand (6×10^6 c.p.m.) was mixed with 75 μg of the poly(A)-containing RNA from neuroblastoma cells in 20 μl of 6 mM NaCl, 6 mM HEPES–KOH, *p*H 7.5, 0.6 mM EDTA. After incubation at 100 °C for 90 s, 5 μl of 0.84 M NaCl were added and the mixture was incubated at 50 °C for 2 h. After incubation, 75 μl of the solution (53 mM Tris-HCl, *p*H 7.5, 6.6 mM dithiothreitol, 6.5 mM $MgCl_2$, 0.64 mM dGTP, dATP, TTP and dCTP) containing 200 units of human placenta ribonuclease inhibitor (Wako Pure Chemicals, Osaka, Japan) and 20 units of reverse transcriptase (Seikagaku Kogyo Co., Tokyo) were added, and the incubation was carried out at 37 °C for 90 min. The mixture was treated with phenol and ethanol-precipitated. The precipitate was subjected to an 8% polyacrylamide sequence gel electrophoresis in the presence of 8.3 M urea[29]. The elongated DNA (corresponding to ~250 nucleotides length) was eluted from the gel and sequenced following the method of Maxam and Gilbert[29].

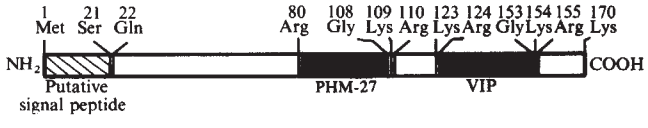

Fig. 2 Schematic representation of the structure of human prepro-VIP/PHM-27. Numbering of the amino acid residues is as in Fig. 1. The putative signal peptide (1–21) is presented by the cross-hatched bar. VIP (125–152) and PHM-27 (81–107) are represented by the closed bars. The amino acid residues at the post-translational processing site are shown.

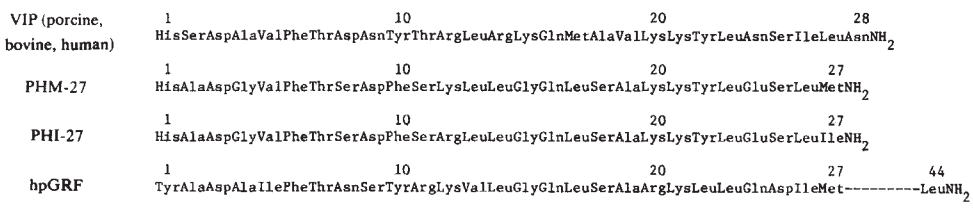

Fig. 3 Comparison of the amino acid sequences of VIP, PHM-27, PHI-27 and hpGRF. The amino acid sequences of human VIP and PHM-27 were deduced from the nucleotide sequence of human prepro-VIP/PHM-27 mRNA described in Fig. 2.

(a peptide (P) having amino-terminal histidine (H), carboxy-terminal isoleucine (I) amide and 27 amino acid residues), a member of the glucagon–secretin family recently isolated from porcine upper intestinal tissue[7]. The amino acid sequence differs from that of porcine PHI-27 only at residues 92 (Arg → Lys) and 107 (Ile → Met). As both these amino acid substitutions can be explained by one-point mutation of the codons, it is plausible to assume that the amino acid sequence at residues 81–107 corresponds to that of human PHI-27. However, we cannot exclude the possibility that authentic PHI-27 may also be found in man and coded for by a different gene from that encoding the VIP precursor. Although it has been shown that a pair of basic amino acid residues are frequent sites for the post-translational processing of hormone precursors[11], the amino-terminus of the peptide is preceded by one basic amino acid residue, Arg, at position 80. A similar example is reported in the cleavage between neurophysin II and the glycopeptide from the precursor protein[14]. The carboxy-terminal methionine residue is followed by a glycine residue and a pair of basic amino acid residues (Lys-Arg), just as with VIP. The glycine residue appears to serve as an amino donor to the carboxy-terminus of the peptide. Therefore, human PHI-27 is thought to be a 27-amino acid peptide (P) having an amino-terminal histidine (H) and a carboxy-terminal methionine (M) amide—PHM-27.

The present results indicate that VIP and PHM-27, two members of the glucagon–secretin family, are synthesized from the common precursor protein, prepro-VIP/PHM-27. Figure 2 shows the schematic representation of the structure of human prepro-VIP/PHM-27. The recent finding that immunoreactive VIP and immunoreactive PHI-27 occur in roughly equimolar concentrations in extracts from a variety of tissues from several species and that VIP-producing tumours also produce PHI-27, have suggested that VIP and PHI-27 might be co-synthesized in the same precursor[15]. On the other hand, immunohistochemical studies have also revealed that there are some differences in the distribution of VIP- and PHI-27-immunoreactive cells in the rat hypothalamus, indicating that VIP and PHI-27 may not coexist in the same neurones[16,17]. Therefore, in the hypothalamic cells the synthesis of VIP and PHI-27 may be regulated at the post-translational processing step. Although VIP is preceded by a pair of basic amino acids (Lys-Arg) in the precursor protein, PHM-27 is preceded by only one basic amino acid (Arg). This difference may be important in the regulation of post-translational processing. Furthermore, both VIP and PHM-27 contain a pair of internal basic amino acid residues (Lys-Lys and Arg-Lys). These could reflect a differential tissue-specific post-translational processing pathway, yielding some 'cryptic' peptides.

Recently, human pancreatic growth hormone-releasing factor (hpGRF, 40 or 44 amino acid residues) has been isolated from a human pancreatic tumour that had caused acromegaly[18,19]. The amino-terminal region of hpGRF has been reported to have a remarkable sequence homology to porcine PHI-27[18,19]. PHM-27 deduced here has two additional residues (positions 12 and 27 in Fig. 3) common to the amino-terminal region of hpGRF.

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A major rearrangement in the *H–2* complex of mouse *t* haplotypes

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A proportion of wild mice carry a chromosome 17 of which a large part is very different from the standard mouse chromosome 17. The affected region is called the *t* complex, and the anomalous chromosomal types are the *t* haplotypes. In combination with various other chromosomes 17, *t* haplotypes can produce crossover suppression, taillessness, transmission distortion, male sterility and lethality early in development[1–3]. The various *t* haplotypes also carry *H–2* specificities which are different from those of other mice[3–8]. This, together with the fact[9,10] that the lethality genes map to both sides of *H–2*, suggests that the major histocompatibility complex is contained within the *t* complex. The lack of recombination between *t* haplotypes and standard chromosomes 17 may be due to large-scale rearrangements. Genetic data support this idea[8–10], in that the *tufted* gene, the *H–2* complex and a group of *H–2*-related genes appear to be in inverted order in *t* haplotypes relative to the standard chromosome 17. The mapping of several *t*-lethal factors close to the *H–2*-related genes in *t* haplotypes[9] suggests that breakpoint(s) may be found here. We have now investigated the major histocompatibility complex of *t* haplotypes by Southern blots using a variety of cloned DNA probes, and find a major rearrangement, specific to the *t* haplotypes, in the *Qa-2,3* region of the complex. This involves the loss of several large homology units, probably including several class I *H–2*-related genes, and the creation of two possible breakpoints.

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