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Perspective: Research Activity of Enteropancreatic and Brain/Central Nervous System Hormones Across Invertebrates and Vertebrates[1]

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SYNOPSIS. During the past two decades there have been rapid advances in our knowledge of the structure and function of the protein hormones in the brain and gastroenteropancreatic system (GEP). Many published articles have highlighted the superfamily of hormonal peptides, specifically, the mechanisms and control of peptide synthesis in neural and non-neural tissues, and gene structure. Here we present an analysis of the annual trends, between 1980 and 1997, of research emphasis on six protein/peptide hormones, as reflected by their individual frequency of publication per year. Although this symposium is focused on the GEP hormones, we provide herein a perspective on the level of research activity of the hormones insulin, glucagon, cholecystokinin, insulin-like growth factor-I and -II, neuropeptide Y and somatostatin in the brain/gut systems throughout the vertebrates and invertebrates. Many publications deal with the evolution of these peptides and their superfamilies, yet as noted in this review, there are relatively few references to these peptides in invertebrates and non-mammalian species. Typically in invertebrates, the number of citations is low and mostly focused on three phyla, the arthropods, mollusks and helminths. Generally, in the vertebrates the smallest number of citations is in the cyclostomes and elasmobranchs. Because most groups of invertebrates and vertebrates have received scant attention, phylogenetic comparisons are limited. Evolutionary information concerning important groups of animals, such as helminths, mollusks, protochordates and cyclostomes, is essential to establish the phylogenetic histories of the hormonal peptides. The challenge to comparative endocrinologists is to examine species in key evolutionary positions in order to gain an understanding of the diversity and function of the hormones and to determine the molecular features that form clues to their phyletic interrelationships and progression.

INTRODUCTION

Insulin, glucagon, cholecystokinin, insulin-like growth factor, neuropeptide Y and somatostatin

The aim here is not to describe the sources, mechanisms of action, gene regulation or function of gastroenteropancreatic (GEP) hormones, but rather to describe the trends in research activity on these peptides in invertebrates and vertebrates, and to suggest further areas of needed research. In this study computer searches were used to access the pertinent comprehensive databases and to elucidate the progress of research in specific scientific areas. The trends in research activity are judged by the number of publications in any one search (explained in a later section). We do not cite individual papers, but rather we refer to the total number of citations or reviews written for each of the peptides within a given time period. It should also be noted at the outset that our knowledge is restricted and it may appear, from our database search, that certain peptides are not found in a particular organism or phylum. This probably reflects not that these peptides are absent, but rather that there is a lack of research dedicated to identifying these peptides in these organisms.

The present review was limited to six representative peptides from the so-called hormonal molecular families and superfam-

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TABLE 1. *Hormones and proteins secreted by the pancreas and gastrointestinal system.**

Hormone/protein	Number of amino acid residues
Hormones and proteins secreted by the mammalian pancreas:	
Glucagon	29
Insulin	51
Islet amyloid polypeptide (IAPP) or ''amylin''	37
Chromogranin A	49
Parathyroid-related protein	141
Somatostatin	14
Pancratic polypeptide	36
Peptide Family	**Hormones**
The families of the related mammalian gastrointestinal peptide hormones:	
Secretin	Enteroglucagon
	Glicentin
	Glucagon
	GIPSecretin
	VIP
	Growth hormone-releasing factor
Gastrin	CCK
	Gastrin
Pancreatic polypeptide	PP
	NPY
	PYY
Tachykinin-bombesin	Neurokinins
	Neuormedins
	Substance K
	Substance P
	Gastrin-releasing peptide
Opioids	Enkephalin
	β-endorphin
	ACTH
	α-melanocyte-stimulating hormone
	dynorphins

* Modified from Norman and Litwack (Norman and Litwack, 1997).

ilies, which are listed in Table 1, that are produced in the GEP system and/or brain/central nervous system/nerve cells (see below) in invertebrates and vertebrates. The peptides that were selected for this study are insulin and insulin-like growth factors-I and -II (IGF-I and -II) of the insulin/insulin-like growth hormone family, glucagon of the secretin family, somatostatin of the somatostatin family, cholecystokinin (CCK) of the gastrin family and neuropeptide Y (NPY) of the pancreatic polypeptide family. Table 1 lists the hormones and proteins secreted by the mammalian pancreas and illustrates the many peptides that are produced in the gastroenteropancreatic (GEP) system (Norman and Litwack, 1997).

TABLE 2. *Years of Discovery of Brain/Gut Peptides. The following table represents the year that the primary amino acid structures of the six peptides were determined for the gastrointestinal system or central nervous system.*

Peptide	GEP	CNS
Insulin	1955	1978
Glucagon	1968	1979
CCK	1975	1976
IGF-I and -II	1978*	1990
Somatostatin	1984	1973
NPY	1982	1982

* Actually determined from plasma.

Of the peptides described in the present review, three were first discovered in the gastrointestinal tract: insulin, CCK and glucagon; IGF was first identified from the plasma: IGF-I and -II; NPY and somatostatin were initially discovered in the brain (Table 2). Subsequent studies have shown these six peptides to be widely distributed and expressed in many tissue types of vertebrate and invertebrate species. However, it is recognized that what may be called in-

sulin in invertebrates is actually insulin-like. In the literature, one will see terms such as ''invertebrate CCK'' or ''brain insulin''; this is often an oversimplification. Molluscan insulin-like peptides and genes are different from mammalian insulin genes (Plisetskaya, 1998). In fact, all insulin- and gastrin-family peptides sequenced from protostomes (arthropods, annelids and mollusks), to date, are not pancreatic type insulins or gut-type-CCKs, but rather insulin-like or CCK-like (Chan *et al.*, 1990; Plisetskaya, 1998). Thus, when it is stated here that insulin is found in both invertebrate and vertebrate phyla, we are referring to the insulin family of peptides and not necessarily the specific pancreatic-type insulin. It is also recognized that the functions of peptides belonging to the same family potentially may differ, and that many of the functions of these peptide-like hormones are still unknown.

There has been a virtual explosion of our knowledge of peptide hormones. Studies of peptide structure, in the spectrum of species, have indicated that portions of a sequence of a given peptide have been conserved during evolution, and such conserved sequences may represent functionally active portions of the molecule (Krieger, 1983). Furthermore, studies have also demonstrated that the same peptide can be expressed in a variety of secretory cells including neurons. Even though the symposium entitled ''A Tribute to Erika M. Plisetskaya: New Insights on the Function and Evolution of Gastoenteropancreatic Hormones'' is centered on GEP hormones, it is important to realize that all of these hormones may be expressed in many other cell types aside from those of the GEP system, and they may have a wide variety of functions. What we know about a particular hormone, its gene or function, has been determined by research activity dedicated to a particular system or animal. Typically, a hormone is thought to have one, or possibly two, functions. For example, when most endocrinologists consider the word ''insulin'', they commonly think of the pancreatic hormone and its control of glucose metabolism, often they do not consider the brain insulin and its putative functions.

DEFINITION OF A HORMONE

The modern concept that hormones regulate both growth and differentiation was clearly enunciated at the turn of the century by Sir Ernest Starling when he introduced the term ''hormone'' during his first Croonian Lecture to the Royal Academy of Physicians entitled ''Chemical Correlation of Body Functions'' (Van Wyk, 1992). Since Bayliss and Starling discovered the first hormone, secretin, from the gastrointestinal tract, our knowledge and definitions of chemical mediators have greatly expanded (Bayliss and Starling, 1902). We now consider that the study of endocrinology encompasses chemical regulation of virtually all biological phenomena (Norris, 1997). Chemical messengers regulate physiological and behavioral functions at all levels of organization including molecular, cellular, developmental, organismic, and ecological. Many scientists now consider the terms chemical messenger and hormone to be synonymous while other scientists consider hormones to be a subset of chemical messengers. In this paper, we will define hormone as any substance that operates at the cellular level, generated either externally or internally, which conveys to that cell a message to stop, start, or modulate a cellular process. As a consequence of molecular and cellular studies during the past decade, many scientists now recognize that messengers such as neurotransmitters and growth factors, by definition, are hormones. Our current understanding of endocrinology is not only profoundly different from that of Starling's time, but it has evolved dramatically over the last ten years.

Though it was once thought that many gastrointestinal hormones were produced only in the GEP, today it appears that most, if not all, of these gastrointestinal hormones are also expressed in other tissues, such as the brain, where they may have other distinct functions such as neurotransmitters (Van Wyk, 1992). The question of the evolutionary origin of brain/gut peptides have aroused great interest and since the 1980's they have opened a wealth of new studies and syntheses. As already stated, we now recognize that the GEP peptides belong to

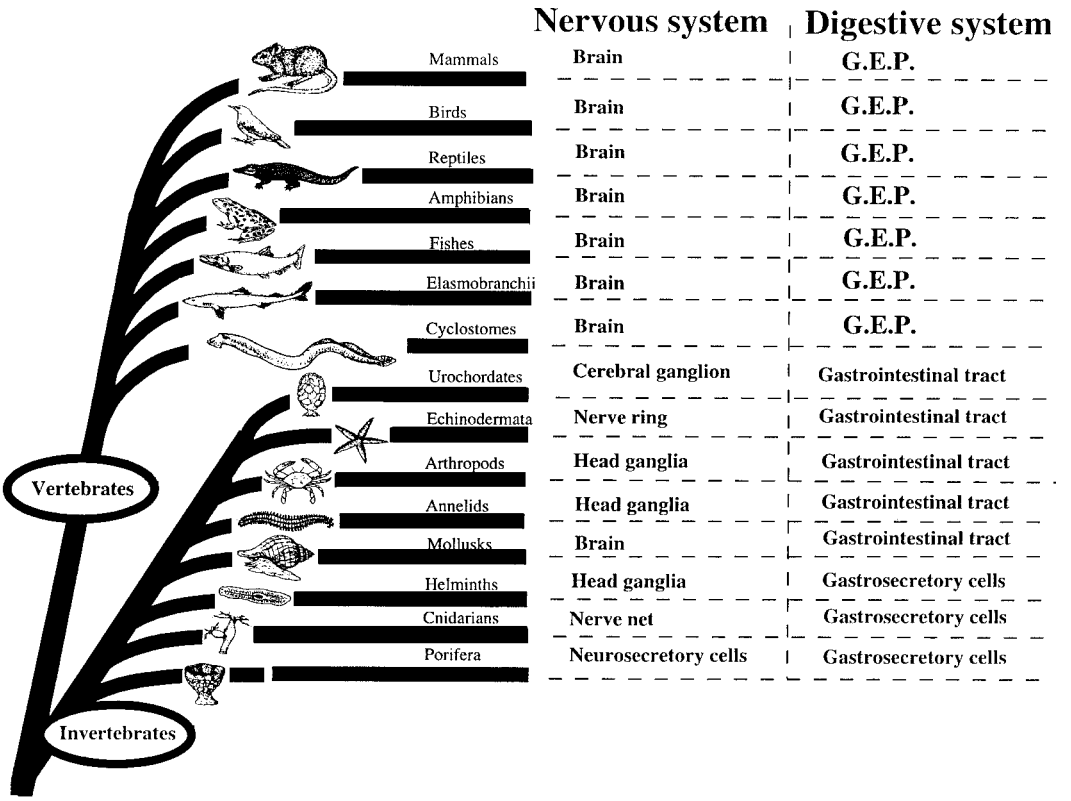

FIG. 1. Schematic evolutionary diagram illustrating the presence/absence and the relative complexity of the nervous and digestive systems of the fourteen classes and phyla detailed in this study.

large families or superfamilies of peptides. As we increase our knowledge about the structures and distributions of these peptides in animal, and even plant organisms, we will gain a better understanding of the evolution of the peptide superfamilies. As stated by Reinecke and Collett (1998), ''our understanding of the mechanism of action of IGFs in mammals will come from our understanding of the evolutionary pathway of the IGF system and the role of the individual components of the growth factor systems in nonmammalian vertebrates and protochordates'' (Reinecke and Collet, 1998).

EVOLUTION OF THE BRAIN AND GEP SYSTEM

It has been suggested that the original cellular messengers in organisms were factors necessary for communication, both for mediation and integration of events within the cells, and for cell-to-cell interactions (Norris, 1997). As multicellular organisms evolved, inter- and intracellular communication and integration became increasingly complex, requiring further evolution of the endocrine and nervous systems. As the complexity of the digestive system increased, there were corresponding increases in hormonal and neuronal control of physiological processes in invertebrates. In Figure 1 the presence, absence and relative complexity of digestive and nervous systems is listed for all phyla considered in this study. In this figure, we use general terms to characterize the occurrence of brain/central nervous system/nerve cells and the gastroenteropancreatic system. The simplest invertebrate form of digestion is found in the sponges (Porifera), which lack a differentiated digestive tract. The cnidarians (hydra, jellyfish, and sea anemones) and helminths (flatworms) have a sac-like digestive

system with a single opening. In the nematodes (roundworms), and all of the more complex animals, an efficient tubular, one-way digestive system has evolved. A GEP system that includes such features as pancreatic islets, bile duct and gastrointestinal system does not appear until the vertebrates (Conlon *et al.*, 1988; Falkmer *et al.*, 1985).

In an evolutionary sense, it is most important to fully appreciate that the relative complexity of various systems, such as the brain, has not evolved in a unidirectional step-wise fashion, but that more complex brains have evolved repeatedly, both among invertebrates and among vertebrates (Bullock, 1993). The vast differences in level of complexity between species within one phylum of invertebrates, between phyla or between agnathans, fishes and mammals, appear to involve more than simple consequences of increased numbers of neurons (Bullock, 1993). New variables, such as neurochemicals, anatomical structures and physiological mechanisms, have been added during the long period of evolution. The peptides, which have known functions in one species, have been co-opted during evolution by other cells or organs and may have different functions. As noted, in Figure 1, there is no true nervous system in the Porifera, however there are sensory and neurosecretory cells present in clusters. The earliest appearance of neurons is noted in the primitive cnidarian and other coelenterate invertebrates, and these neurons secrete both peptide and nonpeptide regulators (Norris, 1997). There are even gross variations in the nervous systems of the invertebrates as shown in Figure 1. Cephalopod molluscs have a highly developed brain, and of course brains of varying degrees of complexity occur in the vertebrates.

EXPRESSION OF PEPTIDES

As shown in Figure 2, insulin, glucagon, somatostatin, NPY, CCK, IGF-I and -II are made by brain/nerve cells and gastrointestinal cells in representative species throughout the invertebrates and vertebrates. The blanks in this figure probably do not reflect an absence of these peptides in the specified species, but rather a lack of research designed to identify these peptides in these organisms. As noted in Figures 3 and 4, the total number of literature citations per year in the invertebrates for any one of the six peptides ranges between 0 to 57, *vs.* the total number of citations in vertebrates which range from 0 to 1,628. Thus, the amount of research conducted on these peptides in invertebrates and non-mammalian species is comparatively low. However, there have been excellent recent reviews on these peptides, some of which are listed in Table 3 and include papers presented at this symposium.

METHOD OF DATABASE SEARCH

For the current study, the computer searches and all subsequent analyses of the total research publications for each neuroactive peptide were limited to the brain and gut/pancreas of the vertebrates and to the brain/nervous tissue and gut of invertebrates. The database utilized for the search was CP-ROM Medline (CD-PLUS) using the procedures described by Myers (Myers, 1994). Medline is generated by the National Library of Medicine, it is international in scope; it is updated monthly, and it includes citations of journal articles, editorials, and even letters to the editor from over 3,600 journals. The time span for the survey was limited to eighteen years (1980–1997). The total numbers of publications per year on vertebrates and invertebrates were obtained initially by ''exploding'' to include all organisms in these categories. The narrowing terms for a particular peptide and year were further added to the query. The terms used for the different classes and phyla of vertebrates and invertebrates were those used by CP-ROM Medline. Some of these terms do not reflect the terms or zoological groupings that are commonly used by biologists in referring to species. As an example, within the CD-PLUS, the term elasmobranchii does not include all chondrichthyes.

The following is the CP-ROM Medline list of terms used for the vertebrates and which we adopted for this survey: Amphibia, Birds, Fishes, Mammals, and Reptiles. The fishes are further divided into Catfish, Cyclostomes, Cypriniformes, Cyprinodontiformes, Eels, Elasmobranchii, Electric Fish, Fishes, Poisonous Fishes, Flatfishes,

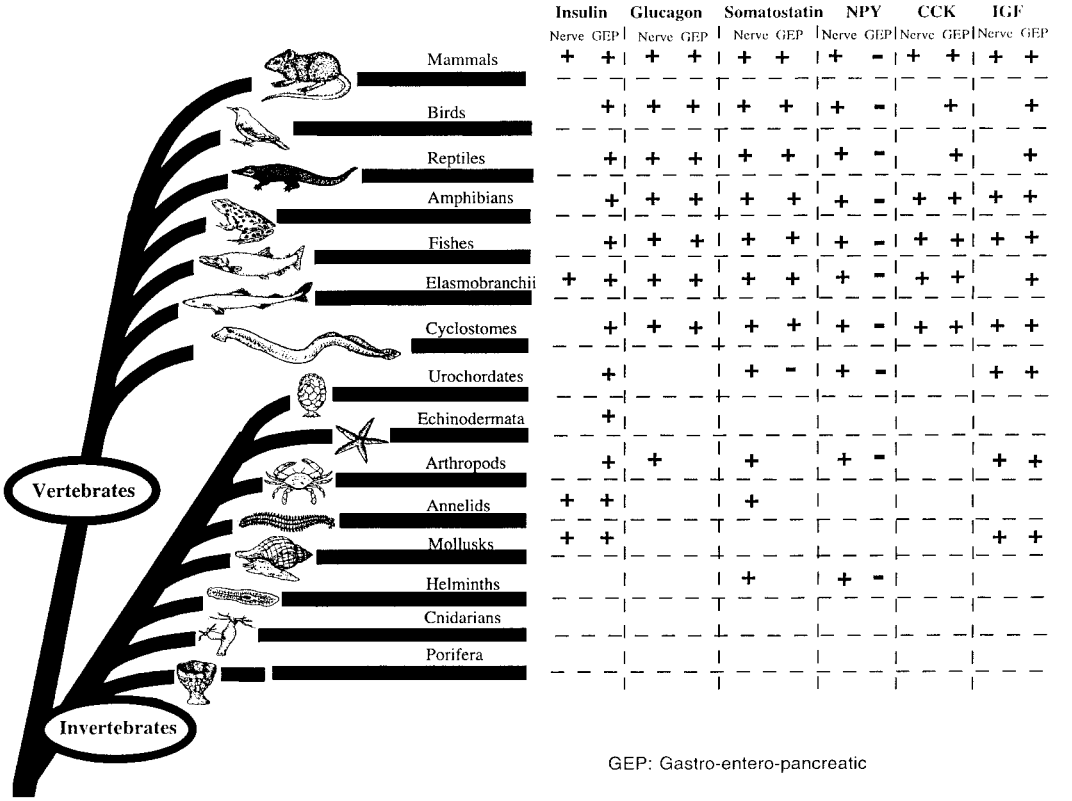

FIG. 2. Schematic evolutionary diagram detailing the presence or absence, as determined by Medline database searches and reviews cited in this study, of insulin, glucagon, somatostatin, NPY, CCK, and IGF in the nervous and digestive systems of the fourteen classes and phyla utilized in this study. (+) indicates the presence of a peptide, (−) indicates the presumed absence of a peptide and (blank) indicates that data were not available.

Perciformes, Salmoniformes and Tuna. As noted, cyclostomes and elasmobranchii are listed under Fishes. In our data base search, we separated out the numbers of citations for elasmobranchii and cyclostomes.

The following is the CP-ROM Medline list of terms used for the invertebrates: Annelida, Arthropods; Bryozoa; Chordata (Urochordata); Cnidaria; Echinodermata; Helminths (Acanthocephala, Nematoda, Platyhelminths, Rotifera); Mollusca; Parasites; Plankton; Porifera; and Protozoa. As noted, urochodordata were listed, but did not include all protochordates, so we excluded this group from our search. We also did not include plankton, parasites, protozoa and bryozoa because of a lack of research citations.

In this paper, we use the term research activity as a correlate of the number of citations found in our searches.

ANALYSIS OF TRENDS OF PEPTIDES FROM 1980 TO 1997

Analysis of the year by year research trends of the six peptides, from 1980 to 1997 in vertebrates and invertebrates are summarized in Figures 3 and 4. The number of citations for insulin in vertebrates is nearly double that of the citations for most of the other peptides, and the number of citations for insulin in vertebrates has remained steady during the past 16 years following a surge in the 1970s (Myers, 1994). In invertebrates, there has been a sustained increase of interest in insulin, as reflected by total publications (Fig. 4). It should be noted that each bar in the histogram repre-

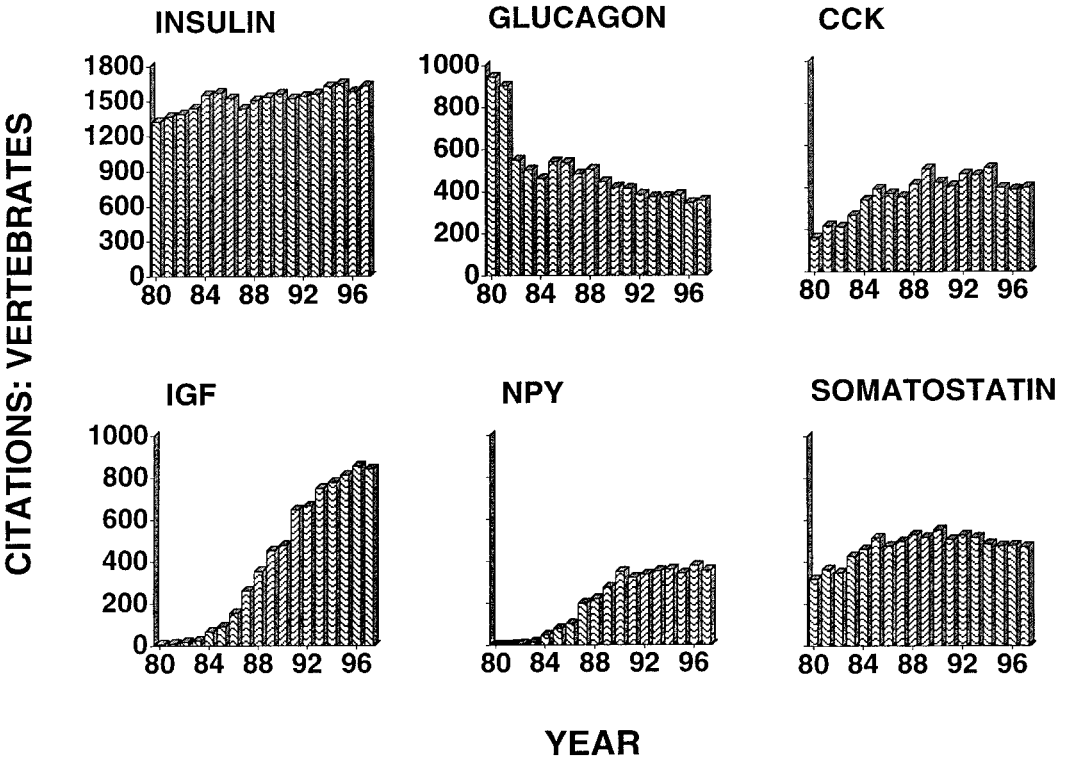

FIG. 3. Total publications per year, from 1980 through 1997, for glucagon, IGF, insulin, somatostatin, NPY and CCK. Data obtained by Medline database searches restricted to the vertebrates.

sents the total number of publications in a given year (abscissa).

Interestingly, most of the insulin citations reported for vertebrates are associated with the GEP system. In contrast, the majority of citations for invertebrates are associated with nerve cells or the nervous system (Fig. 5). Insulin is the only hormone analyzed for the vertebrates that has the most citations associated with the GEP system while the other five hormones have a higher number of citations associated with the vertebrate nervous system. However, in invertebrates, glucagon is the only hormone that has the most citations associated with the GEP system. This research trend probably reflects not only the identification and wide distribution of these peptides in the nervous system and brain, but also the number of neuroendocrinologists *vs.* the number of GEP researchers present in their respective field. Of the six peptides, only glucagon shows a decrease of scientific publications in vertebrates (Fig. 3). In invertebrates, glucagon citations show no clear trend since 1980 (Fig. 4).

INDIVIDUAL TRENDS IN RESEARCH

Insulin

The history of insulin occupies a unique place in the evolution of our understanding of peptide hormones (Table 4). Insulin, released from the vertebrate pancreas, is the primary hormone controlling intermediary metabolism. However, there are other identified roles of insulin such as in the central nervous system which include sympathoexcitation, vagal withdrawal and stimulation of corticotropin-releasing factor (Ferrannini et al. 1999). Table 4, modified from Norman and Litwack (1997), summarizes the notable series of ''firsts'' associated with the development of the chemistry and physiology of this important hormone (Norman and Litwack, 1997). Due to its relatively modest size, insulin was an ideal

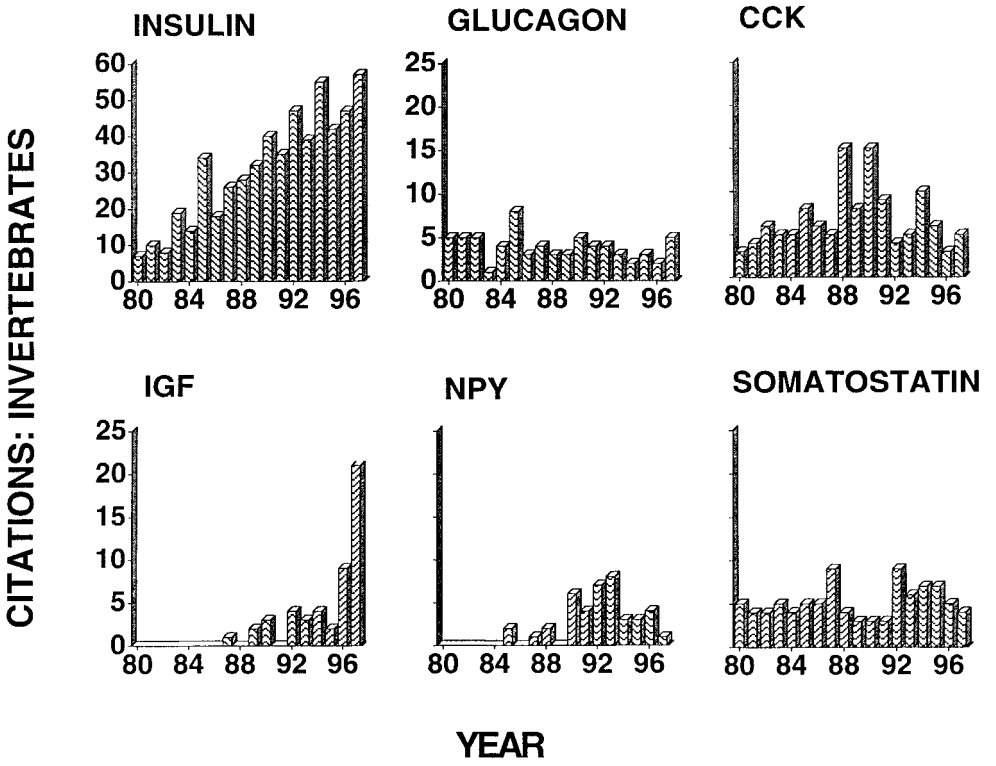

FIG. 4. Total publications per year, from 1980 through 1997, for glucagon, IGF, insulin, somatostatin, NPY and CCK. Data obtained by Medline database searches restricted to the invertebrates.

molecule for pioneering developments in peptide chemistry and amino acid sequencing and in recombinant DNA/gene cloning techniques (Norman and Litwack, 1997).

For vertebrates, the number of publications on insulin from 1980 to 1997 is greatest in mammalian species (Fig. 6). This graph is in log scale so that relative research activity can be noted across the vertebrates. There is greater than fifty times the number of citations utilizing mammals (57,222) compared to the next higher number of citations within the vertebrates, birds with 924 citations. The lowest number of vertebrate citations is from the elasmobranchs (31 citations).

The larger number of research publications for insulin in invertebrates, when

TABLE 3. *Reviews within the last seven years of six GEP/brain peptides.*

Peptide	References
Insulin	(Chan *et al*., 1990; *Chan and Steiner, 2000; *Conlon, 2000; *Planas *et al.,* 2000; Plisetskaya, 1998)
Glucagon	(Drucker, 1990; Holst, 1996; Lefebvre, 1995; *Mommsen, 2000; Plisetskaya and Mommsen, 1996)
Somatostatin	(Conlon *et al*., 1997)
CCK	*(Vigna, 2000)
NPY	(Jessop *et al*., 1992; Larhammar, 1996; Larhammar *et al*., 1993; Palmiter *et al*., 1998; *Silverstein and Plisetskaya, 2000)
IGF-I, -II	(*Chan and Steiner, 2000; Collet *et al*., 1998; DePablo *et al*., 1993; LeRoith *et al*., 1993; *Planas *et al*., 2000; Reinecke and Collet, 1998; Thissen *et al*., 1994)

* Tentative: these are reviews presented in this symposium modified from Norman and Litwack (Norman and Litwack, 1997).

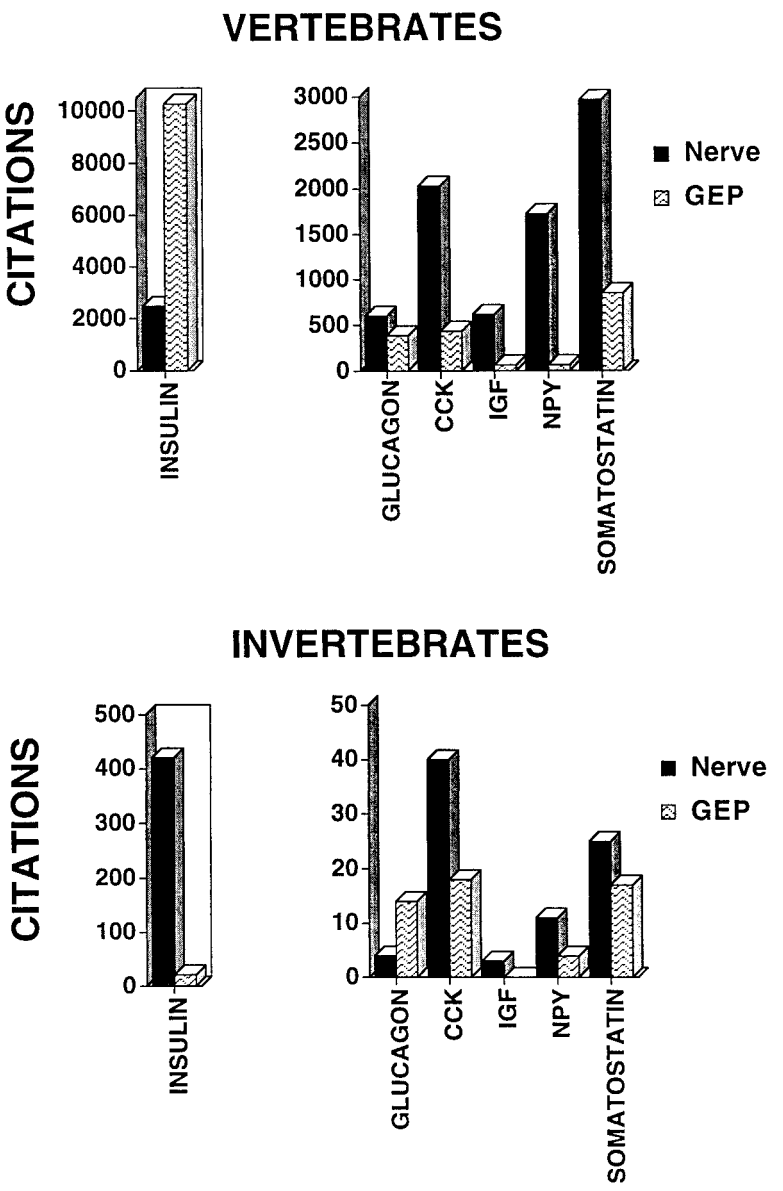

FIG. 5. Total publications from 1980 through 1997 for glucagon, IGF, insulin, somatostatin, NPY and CCK obtained by Medline database searches restricted to either the nervous system/neurons or GEP.

TABLE 4. *History of insulin.*

Year	Event
1869	Discovery of pancreatic islets
1921	Discovery and isolation of insulin (Banting*)
1955	Determination of primary amino acid sequence (Sanger*)
1967	Discovery and structure determination of proinsulin
1969	Determination of three dimensional structure (Crowfoot-Hodgkin*)
1979	Cloning of the insulin gene

* Received Nobel Prize.

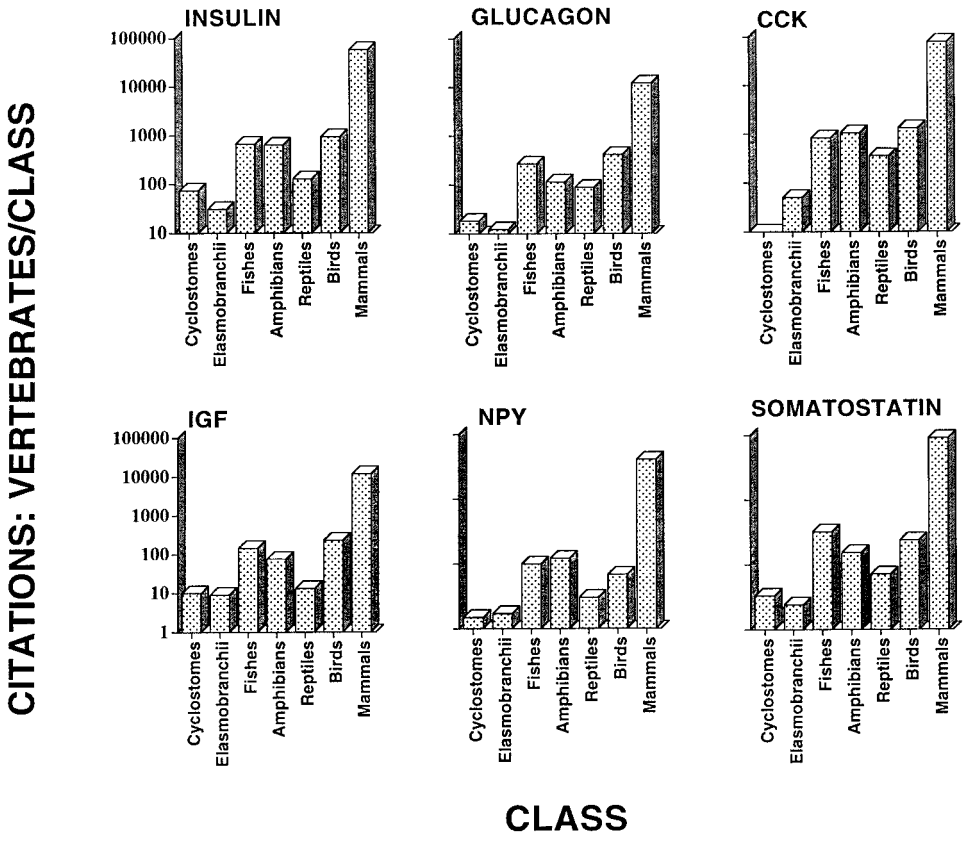

FIG. 6. Total publications from 1980 through 1997 for glucagon, IGF, insulin, somatostatin, NPY and CCK obtained by Medline database searches restricted to individual classes of vertebrates.

compared to the other five peptides, is due mainly to work with the arthropods. As noted in Figure 7, there are more than 250 cited publications about insulin in arthropods since 1980, with a little less than 100 in the helminths and mollusks and less than 15 in the other phyla. When one further refines the search, most of the citations within the helminths are on *C. elegans.*

Glucagon

Among the hormones isolated from the pancreas during the late 1950's, glucagon was one of the first, and subsequently it was purified, sequenced and synthesized. Glucagon is secreted by the α-cells of the pancreas, and it is considered the most potent hepatic glycogenolytic agent known. Furthermore, glucagon is structurally homologous to the family of peptide hormones that includes secretin, vasoactive intestinal polypeptide, gastric inhibitory peptide, enteroglucagon and glicentin (Table 1). Like others of these peptides, glucagon is expressed in the brain stem and hypothalamic neurons (Plisetskaya and Mommsen, 1996).

In vertebrates, the level of research activity on glucagon peaked in the early 80's and then declined (Fig. 3). As in the case of insulin, most of the glucagon research activity has been cited in mammals with 11, 987 citations (Fig. 6). Next to mammals, birds have 411 and fishes have 265 citations. The smallest number of citations within the vertebrates for glucagon is for the elasmobranchii. Citations of glucagon research in invertebrates (Fig. 4) are few. Most of the citations on glucagon research, as in the case of insulin, has been cited in arthropods 35 citations; this may be compared to 14 citations in the helminths (Fig. 7).

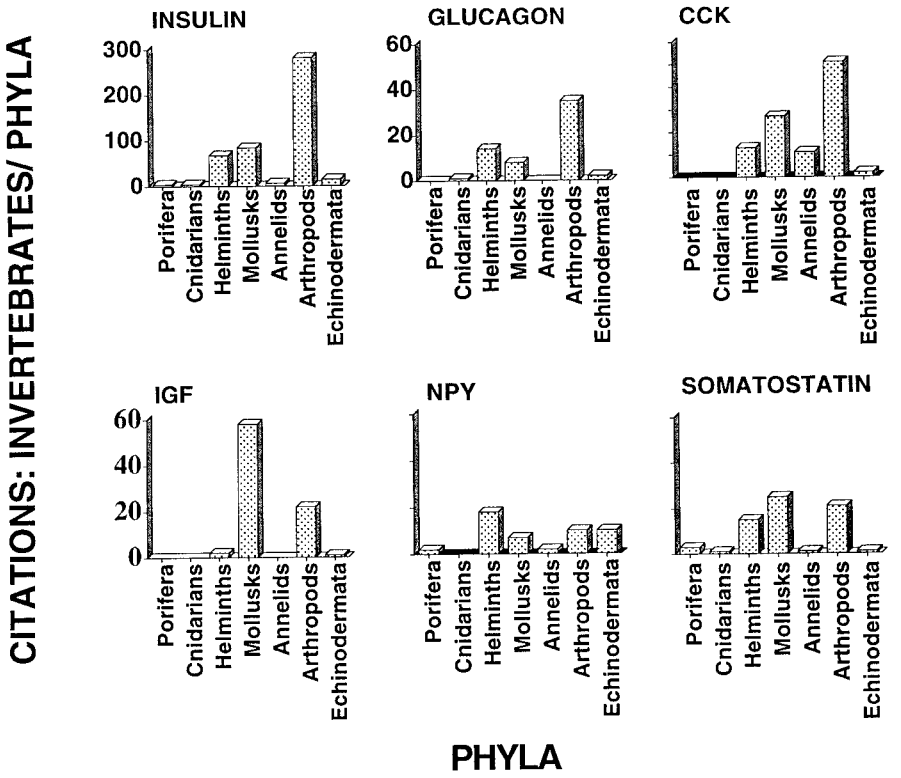

FIG. 7. Total publications from 1980 through 1997 for glucagon, IGF, insulin, somatostatin, NPY and CCK obtained by Medline database searches restricted to individual phyla of invertebrate taxa.

CCK

CCK was originally described and isolated in mammals as an intestinal hormone, but later it was found in the central and peripheral nervous systems of vertebrates (Crim and Vigna, 1983; Vigna, 1985). CCK is expressed principally in the duodenum, jejunum and in the central nervous system, as well as in the nerves of myenteric plexus of the intestine and the nerves of the urinary bladder and uterus in mammals.

The major functions of the octapeptide CCK, as a GI hormone, include the stimulation of synthesis and secretion of pancreatic enzymes as well as gallbladder contraction in mammals. Unfortunately, there have been no recent reviews of CCK research. However, in an earlier review it was shown that CCK peptides have a long evolutionary history (Vigna, 1985). Immunological evidence indicates that CCK-like molecules can be traced back through the invertebrates at least as far as the Cnidaria (Crim and Vigna, 1983). However, the immunological data also suggest that these invertebrate CCK-like peptides are substantially different in structure from vertebrate CCKs (Vigna, 1985).

In vertebrates and invertebrates, the research activity on CCK peaked in the late 1980s and early 1990s and then slightly declined (Figs. 3, 4). In vertebrates, as for insulin and glucagon, most of the research activity has been cited in mammals (7,845) from 1980 to 1997 (Fig. 6). Next in order of citations in vertebrates is birds with 132 citations. The smallest number of vertebrate citations for CCK are from the elasmobranchii and cyclostomes with 5 and 0 citations, respectively. In invertebrates, the highest number of citations was in the arthropods with 51 citations (Fig. 7).

IGF

The primary structures of insulin-like growth factor-I and -II were determined

from human plasma (Rinderknecht and Humbel, 1978*a*, *b*). The insulin-like growth factors are polypeptides that are structurally similar to insulin (Reinecke and Collet, 1998). As with the other peptides, not only is IGF (I and II) expressed in the GEP system but also in neuronal and glial cells. The insulin-like growth factors are major regulators of growth and development in mammals.

In both vertebrates and invertebrates, the research activity on IGF has been steadily increasing since the early 1980s. As with all the other peptides, most of the research activity on IGF has been cited in mammals with 11,977 citations (Fig. 6). The two next closest groups to mammals are birds with 229 citations and fishes with 146 citations. The smallest number of citations within the vertebrates is from the elasmobranchii with 10 citations. In invertebrates, the highest number of citations was from mollusks with 56 citations (Fig. 7).

Neuropeptide Y-related peptide

Neuropeptide Y (NPY) is a member of the pancreatic polypeptide (PP) family. Other members of this family include pancreatic polypeptide (PP), peptide tyrosine-tyrosine (PYY), fish pancreatic peptide Y (PY), and peptide methionine-tyrosine (PMY), isolated from the sea lamprey (Conlon *et al.*, 1991; Larhammar, 1996; Larhammar *et al.*, 1993). The name of this family was derived from the first member of the family to be discovered and named pancreatic polypeptide. PP was originally isolated from the chicken pancreas and was actually a byproduct of insulin purification (Larhammar *et al.*, 1993). The members of the PP family have a wide anatomical distribution within organisms, and accordingly, a diverse range of physiological functions.

In vertebrates, as for IGF, the number of citations of NPY research has been steadily increasing since the early 1980s (Fig. 3). This increase of research activity probably reflects the wide distribution of NPY in regions of the brain and the diverse range of its physiological functions such as feeding behavior, central control of cardiovascular function, memory processing and the reproductive system (Myers, 1994). In invertebrates, NPY research activity peaked in 1992 and 1993 and decreased in the late 1990s (Fig. 4), although the number of citations is very low, ranging from 0 to 18. In vertebrates, as for the other peptides, most of the research activity has been cited in mammals (4068) from 1980 to 1997 (Fig 6). Next in number of citations is amphibians with 121. The smallest number of citations within the vertebrates for NPY is for the elasmobranchii with 17 citations and cyclostomes with 15 citations. In invertebrates, the largest number of citations was for the helminths with 18 (Fig. 7).

Somatostatin-14

The neurohormone somatostatin-14 (SS-14) was first isolated from the ovine hypothalamus and it was shown to inhibit release of pituitary growth hormone (Brazeau *et al.*, 1973). Subsequent studies have shown that somatostatin is widely distributed in vertebrate neuroendocrine tissues as reviewed in (Conlon, 1990).

In vertebrates, the research activity on somatostatin has been steady and at the same level since the mid 1980s (Fig. 3). In invertebrates, somatostatin research activity has increased slightly in the late 1990s (Fig. 4), although the number of citations is very low ranging from 1 to 25 (Fig. 7). In vertebrates, as for the other peptides, most of the research activity has been cited in mammals (9,033) from 1980 to 1997 (Fig. 6). Next to the mammals are the fishes, with 324 citations. The smallest number of citations within the vertebrates on somatostatin is for the elasmobranchii with 24 citations and cyclostomes with 33 citations. In invertebrates, the highest number of citations was for the mollusks with 25 citations (Fig. 7).

SUMMARY AND FUTURE TRENDS

For a better insight into the molecular evolution of brain/gut hormonal peptides, and their genes and receptors, it will be necessary to isolate and characterize these hormones not only from the vertebrates, but also from the protochordates (amphioxus and ascidians) and invertebrates. Many papers deal with the evolution of these peptides and their families, yet as shown by

citation frequency, there are still relatively few studies of these peptides in invertebrates and non-mammalian species. Typically in invertebrates, citation numbers are low and mostly focused on three phyla, the arthropods, mollusks and helminths. In vertebrates, the smallest number of research citations on these peptides are commonly in the cyclostomes and elasmobranchs. Because most groups of invertebrates and vertebrates have received scant appropriate investigative attention, phylogenetic comparisons are difficult or impossible. Data concerning key evolutionary invertebrate and vertebrate groups, the protochordates, chondrichthyes and cyclostomes (Agnathans), are essential to establish evolutionary patterns of the peptides.

It is important to recognize the limitations of the data presented in this survey. As an example, in this paper we did not survey the interrelated superfamily of peptides such as the insulin/IGF superfamily. Instead we have presented the data for two different peptides. There were citations listed for insulin-like IGF in invertebrates, but many of these were probably related to the insulin-like peptides (ILP) such as bombyxin in arthropods and MIP in mollusks (Chan *et al.*, 1990). Chan and colleagues have suggested that ILP may represent an intermediate form linking the IGF genes with an ancestral insulin gene. Based on their detailed findings, these authors have proposed that insulin and IGFs may have evolved from a common ancestral form and that a separate IGF emerged at a very early stage in vertebrate evolution from an ancestral insulin-type gene by gene duplication and divergence.

The continuing challenge to comparative endocrinologists is to examine species in key evolutionary positions to gain an understanding of the diversity and adaptive functions of the hormones and to determine their molecular evolution. The families of hormonal peptides and the functional diversity of these hormones is due not only to gradual molecular mutational events, but also to parallel evolution of their receptors and to the varying localization of these receptors in different organs and tissues. The lack of information for such a synthesis limits the utility of the analyses that have been done to date. Progress in this field has stemmed from major contributions from individual researchers such as Dr. Erika Plisetskaya and others who have presented reviews in this conference (see Table 3). They have laid the framework for much of our current understanding about the evolution of gastroenteropancreatic (GEP) hormones and metabolic/growth control systems. As an example, much has been learned about pancreatic hormones in teleosts from Erika Plisetskaya and about their structures from Michael Conlon and colleagues.

The information so far gathered by researchers in the field of comparative endocrinology show a remarkable degree of unity in the organization of the families of peptide hormones and diversity in their cellular sources. Now there is a strong need for new research aimed to demonstrate the differences and similarities of brain/gut hormones in structure and action between major taxa of different levels of complexity. Most important for those of us who work on brain or GEP hormones is to have a more complete understanding of the distribution and actions of these hormones and their adaptive value.

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