VIP: Molecular Biology and Neurobiological Function

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Abstract

In the mammalian brain, a major regulatory peptide is vasoactive intestinal peptide (VIP). This 28 amino acid peptide, originally isolated from the porcine duodenum, was later found in the central and peripheral nervous systems and in endocrine cells, where it exhibits neurotransmitter and hormonal roles. Increasing evidence points to VIP's importance as a mediator or a modulator of several basic functions. Thus, VIP is a major factor in brain activity, neuroendocrine functions, cardiac activity, respiration, digestion, and sexual potency. In view of this peptide's importance, the mechanisms controlling its production and the pathways regulating its functions have been reviewed. VIP is a member of a peptide family, including peptides such as glucagon, secretin, and growth hormone releasing hormone. These peptides may have evolved by exon duplication coupled with gene duplication. The human VIP gene contains seven exons, each encoding a distinct functional domain on the protein precursor or the mRNA. VIP gene transcripts are mainly found in neurons or neuron-related cells. VIP gene expression is regulated by neuronal and endocrine signals that contribute to its developmental control. VIP exerts its function via receptor-mediated systems, activating signal transduction pathways, including cAMP. It can act as a neurotransmitter, neuromodulator, and a secretagog. As a growth and developmental regulator, VIP may have a crucial effect as a neuronal survival factor. We shall proceed from the gene to its multiple functions.

Index Entries: Vasoactive intestinal peptide; gene expression; neuropeptides; neurotransmitters; neuromodulators; neuronal survival.

Introduction

The 28 amino acid peptide, VIP, is widely distributed in the central and peripheral nervous system. This widespread distribution is correlated with VIP's involvement in a wide variety of biological activities including systemic vasodilation, increased cardiac output, bronchodilation, hyperglycemia (*see* Said and Mutt, 1970, 1988), smooth muscle relaxation (Piper et al., 1970), some differential effects on secretory processes in the gastrointestinal tract (Said, 1982; Barbezat and Grossman, 1971; Makhlouf et al., 1978), and gastric motility (Reid et al., 1988). Moreover, VIP induces glycogenolysis in liver (Kerins and Said, 1973) and cerebral cortex (Magistretti et al., 1981).

In the reproductive system, VIP has been suggested as a modulator of vascular reactivity for penile erection (Ottesen et al., 1984; Dixson et al., 1984; Carati et al., 1988). In related studies, we found that VIP can potentiate sexual behavior in rats with reduced masculine functions (Gozes et al., 1989). VIP also influences cervical blood flow (Allen et al., 1988), and produces increases in vaginal blood flow and fluid secretion (Fahrenkrug et al., 1988). Moreover, VIP-mRNA can be found in the ovaries (Gozes and Tsafriri, 1986), where it stimulates oocyte maturation and induces steroidogenesis and cAMP production (Tornell et al.,1988).

The systemic vasodilation effects of VIP may be found in the brain as well. VIP-containing structures have been identified by light and electron microscopy techniques as being closely associated with blood vessels in the brain (Said, 1982; Lee et al., 1984). In addition, VIP has induced relaxation of cerebral blood vessels (Lee et al., 1984), supporting the hypothesis that a VIP-like substance is the transmitter for vasodilation in the brain (Said, 1982). Indeed, electrical stimulation of cholinergic and VIPergic sphenopalatine ganglion neurons, which innervate cerebral blood vessels, caused an increase in cerebral blood flow (Seylaz et al., 1988).

In the nervous system, in addition to its presence in neurons (Besson et al., 1979; Morrison et al., 1984) and its electrically induced release from them (Said, 1984), further evidence supporting a possible neuronal messenger function for VIP includes its localization in synaptosomal preparations (Giachetti et al., 1977) and potassium-induced release from these preparations (Emson et al., 1978). Furthermore, VIP has been localized in synaptic vesicles in the rat hypothalamus (Emson et al., 1978), and VIP-like immunoreactivity was found in large dense core vesicles in nerve terminals innervating exocrine glands (Johansson and Lundberg, 1981; Pelletier et al., 1981). VIP release is induced by electrical field stimulation in a different fashion from acetylcholine (Agoston et al., 1988b), probably via different voltage-sensitive calcium channels (Agoston et al., 1989).

Below, we shall proceed in a step-wise manner from the VIP gene to biological function, reviewing the expanding literature in general, but with a concentration on studies related to our research interests.

The VIP Gene Family: Multiple Genes and Multiple Peptides

The well-characterized VIP molecule (from porcine intestine) shows structural similarities with other gastrointestinal hormones (Said and Mutt, 1970; Said, 1986; Said and Mutt, 1988), such as secretin, glucagon, gastric inhibitory peptide (GIP), PHI or PHM (27 amino acid peptides, having *N*-terminal histidine and C-terminal isoleucine-amide = PHI in the pig and rat; and C-terminal methionine-amide = PHM in the human) (Tatemoto and Mutt, 1980; Nishizawa et al., 1985; Itoh et al., 1983; Bloom et al., 1983), and growth hormone releasing hormone (GHRH) (Guillemin et al., 1982). Other VIP-related peptides, such as helodermin, are still being discovered (Robberecht et al., 1985). The amino acid sequences of the various members of the VIP peptide family are depicted in Fig. 1; identical amino acids that are shared with VIP are shown in bold letters (adapted from Said, 1986). This family of peptides is found in the gut, as well as in the central and peripheral nervous systems, and it is likely that they all act not only as hormones, but also as neurotransmitters or neuromolators.

To study VIP gene regulation in depth, the human cDNA encoding the precursor to VIP has been isolated (Itoh et al., 1983, Bloom et al., 1983). The cDNA sequence revealed that the VIP-mRNA encodes also for PHM in humans and PHI in rats (Nishizawa et al., 1985). We have further isolated the human VIP gene using synthetic, sequence specific, oligodeoxynucleotides as detection probes (Gozes et al., 1984a,b; Bodner et al., 1985; Gozes et al., 1986). The human VIP gene was found to contain seven exons (Fig. 2). Each of these exons encodes a distinct functional domain of the VIP precursor or its mRNA. The gene spans 8837 base pairs (bp). Exon 1 of 165 bp consists of the 5' untranslated region of the mRNA, exon 2 of 117 bp encodes the signal peptide, exon 3 of 123 bp encodes an *N*-terminal peptide, exon 4 of 105 bp encodes PHM, exon 5 of 132 bp encodes VIP, exon 6 of 89 bp encodes the C-terminal peptide, and exon 7 of 723 bp consists of the 3' untranslated region of the mRNA (Bodner et al., 1985; Tsukada et al., 1985; Gozes et al., 1986; Linder et al., 198 ;Yamagami, et al., 1988).

The discovery of VIP and PHM or PHI sequences on the same gene and mRNA suggests cosynthesis of the two peptides in the same tissue. However, one does not always find VIP and PHI in the same cell (Beinfeld et al., 1984); hence, alternative processing of the nuclear precursor RNA could take place. It is intriguing that the 3' splice sites of the VIP and the PHM exons contain an identical stretch of nine nucleotides. At the cDNA level, the 3' splice sites contain the same stretch of six nucleotides that are identically spliced (Bodner et al., 1985; Itoh et al., 1983). These sequence homologies,

VIP

HIS SER ASP ALA VAL PHE THR ASP ASN **TYR THR ARG LEU ARG** LYS **GLN**

MET **ALA** VAL **LYS LYS TYR LEU ASN SER ILE LEU ASN**-NH_2

PHI

HIS ALA **ASP** GLY **VAL PHE THR** SER ASP PHE SER **ARG LEU** LEU GLY **GLN**

LEU SER ALA **LYS LYS TYR LEU** GLU **SER** LEU ILE-NH_2

SECRETIN

HIS SER ASP GLY THR **PHE THR** SER GLU LEU SER **ARG LEU ARG** ASP SER

ALA ARG LEU GLN ARG LEU **LEU** GLN GLY LEU VAL-NH_2

GHRH

TYR ALA **ASP ALA** ILE **PHE THR** ASN SER **TYR** ARG LYS VAL LEU GLY **GLN**

LEU SER ALA ARG **LYS** LEU **LEU** GLN ASP ILE MET SER .....

GLUCAGON

HIS SER GLN GLY THR **PHE THR** SER ASP **TYR** SER LYS TYR LEU ASP SER

ARG ARG ALA GLN ASP PHE VAL GLN TRP LEU MET **ASN** THR

GIP

TYR ALA GLU GLY THR **PHE** ILE SER ASP **TYR** SER ILE ALA MET ASP LYS

ILE ARG GLN GLN ASP PHE VAL **ASN** TRP LEU **LEU** ALA .....

HELODERMIN

HIS SER ASP ALA ILE **PHE THR** GLN GLN **TYR** SER LYS **LEU** LEU ALA LYS

LEU **ALA** LEU GLN **LYS TYR LEU** ALA **SER ILE LEU** GLY .....

Fig. 1. Amino acid sequence of peptides in the VIP family. Amino acid identities with VIP are in bold. The VIP sequence is found in human, porcine, bovine, and rat. The PHI sequence is the porcine sequence. The secretin sequence is the porcine and bovine sequence. The GHRH (growth hormone releasing hormone) is the human sequence. The glucagon is the porcine and human sequence. The GIP (gastric inhibitory peptide) is the porcine sequence (adapted from Said, 1986).

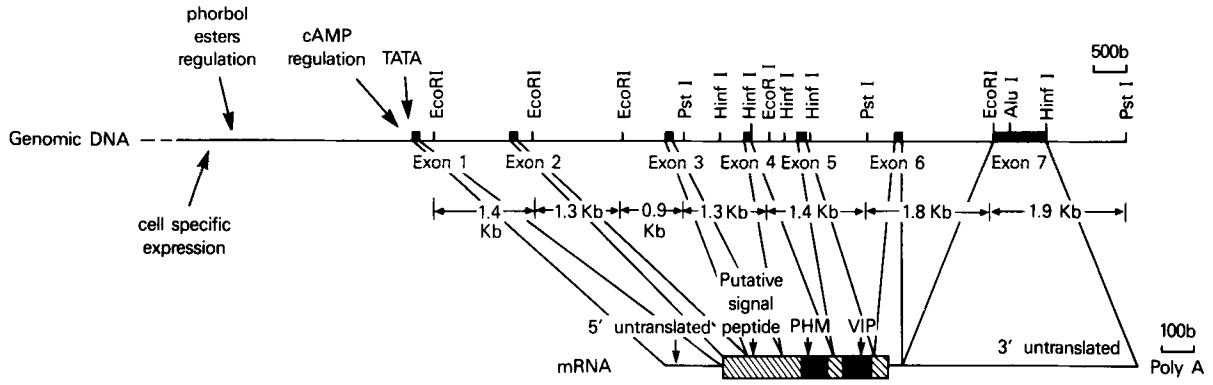

Fig. 2. A schematic representation of the human VIP gene structure.

probably representing conserved areas resulting from exon duplication, may facilitate exon exclusion events during the RNA splicing process. Alternatively, differential protein processing may occur, resulting in cells expressing either VIP or PHI. RNA blot hybridization experiments (e.g,. Gozes et al., 1987d; Linder et al., 1987), as well as *in situ* hybridization studies (Card et al., 1988, Gozes et al., 1989a; Baldino et al., 1989), point out that all brain cells synthesizing VIP-mRNA also contain PHM/I-mRNA. Thus, the possible differential regulation of these two peptides could be at the protein processing level.

Unlike most of the human genes previously sequenced, the VIP gene has an intron within the region coding for the 3' untranslated area of the mRNA (Bodner et al., 1985; Tsukada et al., 1985; Gozes et al., 1986). The significance of such an intron may reside in its ability to control VIP gene activity since the 3' region of genes, in fact, may be involved in splicing events and regulation of transcription (Birnstiel et al., 1985). Furthermore, introns have been shown to contain enhancer sequences, such as in the case of the immunoglobulin gene (Bergman et al., 1984) and the growth hormone gene (Moore et al., 1985).

The 1929 bp sequence of the 5' flanking region of the human VIP/PHM gene was also determined (Yamagami et al., 1988). Four TATA-box sequences were present at 28, 145, 772, and 900 bp upstream of the cap site. Primer extension, exon mapping, and mung bean nuclease mapping analyses revealed that only the TATA box sequence 28 bp upstream of the cap site is the promoter that is inducible by cAMP in human neuroblastoma cells (Yamagami et al 1988). Previous experiments have shown that in human neuroblastoma cells (Hayakawa et al., 1984), cAMP induces a substantial increase in VIP immunoreactivity, which is regulated at the transcriptional level. Indeed, when neuroblastoma cells were grown for 48 h in the presence of 1 m*M* dibutyryl cAMP, the synthesis of the pre-proVIP-PHM/I-mRNA was stimulated 11-fold. The quantity of the mRNA was determined both by hybridization with cloned cDNA, as well as by cell-free translation assays (Hayakawa et al., 1984). Transcription of pre-pro VIP-PHM/I-mRNA in isolated nuclei was observed in cAMP-induced cells, but not in non-induced cells. At the DNA level, no change was observed in VIP gene structure or gene dosage upon treatment with cAMP. All these results suggest an effect of cAMP on VIP gene transcription (Hayakawa et al., 1984). The cAMP effect can also be obtained with forskolin, a diterpene that activates adenylate cyclase (Pruss et al., 1985).

In addition, an increase in protein kinase C activity induced by the phorbol ester, 12-0-tetradecanoyl-phorbol-13 acetate (TPA), may enhance VIP synthesis in adrenal chromaffin cells (Pruss et al., 1985). This effect of phorbol ester is specific for VIP and not a general effect of elevation of peptide synthesis. Indeed, enkephalin synthesis was not affected by the phorbol esters in the same chromaffin cells (Pruss et al., 1985). In human neuroblastoma cells, phorbol esters increased the pre- pro-VIP/PHM mRNA by about fourfold (Ohsawa et al., 1985). This increase was independent of cAMP regulation since the intracellular cAMP level was essentially unaffected by phorbol esters. As in the case of cAMP activation of VIP production, the VIP/PHM gene dosage was unchanged by phorbol esters. In the presence of both cAMP and phorbol esters, the VIP/PHM mRNA level increased by about 36-fold, which is about 3- to 10-fold the amount of increase obtained in the presence of either inducer alone. Thus, there is a synergistic stimulation of VIP/PHM gene expression by cAMP and phorbol esters through independent pathways (Ohsawa et al., 1985). These results suggest that a variety of factors activating either a cAMP-dependent protein kinase or protein kinase C can affect VIP gene transcription mediated via different sites on the gene.

cAMP-regulated transcription of the human VIP gene is dependent upon a 17 bp DNA element located 70 bp upstream from the transcription initiation site (Tsukada et al., 1987) (Fig. 2). This enhancer is similar to sequences in other genes known to be regulated by cAMP, e.g., somatostatin, tyrosine hydroxylase, leu-enkephalin; (Hyman et al., 1988) alpha chorionic gonadotrophin and phosphoenolpyruvate carboxykinase (*see* Fink et al., 1988) and to sequences in several viral enhancers, such as adenovirus DNA (Lin and Green, 1988) and the c-*fos* oncogene (Rauscher et al., 1988). Fink et al. (1988) have demonstrated that the VIP regulatory element is an enhancer that depends upon the integrity of two CGTCA sequence motifs for biological activity. Mutations in either of the CGTCA motifs diminished the ability of the element to respond to cAMP.

Enhancers containing the CGTCA motif from somatostatin and adenovirus genes compete for the binding of nuclear proteins from C6 glioma and PC12 cells to the VIP enhancer, suggesting that CGTCA-containing enhancers interact with similar transacting factors. The binding protein for the cAMP-regulated enhancer was found to be a 43,000 dalton protein, in the case of the somatostatin gene promoter (Montminy and Bilezikjian, 1987). This protein is phosphorylated in vitro when incubated with the catalytic subunit of cAMP-dependent protein kinase (Montminy and Bilezikjian, 1987). Moreover, microinjection of the catalytic subunit of the cAMP-dependent protein kinase into cells containing the cAMP-responsive VIP element fused to a bacterial reporter gene, resulted in the activation of this promoter and expression of the reporter gene (Riabowol et al., 1988).

As for the phorbol ester responsive site on the VIP gene, new evidence (Waschek et al., 1988) suggests that it is located at least 2500 bp 5' upstream of the promoter (Fig. 2). The expression of a transfected plasmid containing 5200 bp of the 5' regulatory DNA sequences of the human VIP gene attached to the coding sequences of the reporter gene was compared with endogenous VIP expression in subclones of the human neuroblastoma cell line, SK-N-SH. The reporter gene activity reflected closely the differences in basal VIP expression and the changes in response to phorbol esters. Deletion of 2700 bp of the most upstream sequences resulted in an 80% reduction in PMA stimulation of the reporter gene activity in the subclone SH-SY-5Y. The same 2700 bp fragment seems to be responsible for basal VIP gene expression in the SH-IN subclone, but not in the SH-SY-5Y subclone. These data indicate that important cell-type-specific transcription regulatory sequences reside further than 2500 bp upstream from the VIP transcription start site (Fig. 2).

The interactions between nuclear proteins and the promoter region of the VIP gene were analyzed for ontogenic and tissue specificity (Giladi and Gozes, in preparation). Tissue-specific nuclear extracts from rats of different ages were electrophoresed on SDS polyacrylamide gels, transferred to nitrocellulose filters, and probed with a nick translated 5000 bp DNA fragment containing the VIP gene promoter and 3500 bp 5' upstream of the promoter region. The ability to detect DNA binding proteins by blotting procedures was demonstrated using stringent hybridization and washing conditions (Bowen et al., 1980; Miskimins et al., 1985; Silva et al., 1987). Under these conditions, we found that the promoter region of the VIP gene interacts selectively with specific proteins that appear when the VIP gene is induced. In addition, the c-*fos* oncogene complex, which binds to the phorbol ester responsive DNA element, may be associated with the regulation of neuropeptide synthesis during brain development (White and Gall, 1987; Gubits et al., 1988). Thus, it is likely that the VIP gene is regulated by the c-*fos* protein complex, via phorbol ester activation. In addition, two sites of control by cAMP are possible, one is direct (*see above*), and the latter, an indirect site modulated by c-*fos* since the c-*fos* gene is also activated by cAMP (Buscher et al., 1988; Rauscher et al., 1988).

In its overall architecture, the VIP gene is similar to the genes coding for other peptides in the family, such as the glucagon gene (Bell et al., 1983; Heinrich et al., 1984) and the growth hormone releasing hormone (GHRH) gene (Mayo et al., 1985). The exon-intron organization of these genes indicates that each exon encodes a functionally-distinct region of the protein precursor and the respective mRNA. In the case of the mammalian glucagon gene, the presence of three exons possessing internal homology (i.e., the glucagon and the two glucagon-like peptides coding exons) may be a consequence of tandem exon duplication (Bell et al., 1983) since the anglerfish cDNA encodes only glucagon and one glucagon-like peptide (for review, *see* Bell, 1986). Recently, it was discovered that the anglerfish expresses two different genes for prepro-glucagon, each encoding one glucagon and one glucagon-like peptide (Nichols et al., 1988). The overall similarity of the genes coding for the peptides of the VIP family actually suggests an evolution from a common ancestral gene. However, the major differences observed among the peptide members of the family may result from a complicated pattern of evolution that combines exon duplication, coupled with gene duplication (Bell, 1986). Such a process may reflect the physiological importance of this peptide family in higher vertebrates. The segregation of exons into distinct functional domains is not always the case for peptide hormone coding genes. An example of the opposite case is the family of genes coding for the opiate peptides, where most peptides are encoded together on one exon (Krieger et al., 1983).

Despite the overall similarity in the structure of the VIP-glucagon GHRH gene family, they do not cross-hybridize. Thus, in Southern blot analysis (Southern, 1975; Bodner et al., 1985; Gozes et al., 1986), using the VIP gene probe, only DNA fragments corresponding to the authentic VIP gene were detected, as a single copy in the human haploid genome (Gozes et al., 1986; Tsukada et al., 1985). At the chromosomal level, these genes are segregated as well; the glucagon gene is located on chromosome 2 (Tricoli et al., 1984), and the GHRH gene is located on human chromosome 20 (Mayo et al., 1985). Using rodent x human somatic cell hybrids, we localized the VIP gene to human chromosome 6 (6p21–6qter) (Gozes et al., 1987a). Furthermore, using *in situ* hybridization, we have localized the VIP gene more precisely to 6q24 (Gozes et al., 1987b). Our results were later corroborated by Gotoh et al. (1988), assigning the VIP gene to 6q26–q27. This latter region is proximal, but different from 6q24. The reasons for this discrepancy are uncertain but might be owing to differences in detection procedures or in the races

(Asians, 6q26–27; whites, 6q24) or individuals analyzed.

As for expression of the various genes in the family, they are transcribed in specific cells, suggesting different control regions. Indeed, a major site of expression for the glucagon gene is in alpha cells of pancreatic islets. This expression is controlled by a 300 bp of the 5'-flanking region of the rat glucagon gene. Three transcriptional control elements were discovered, and the proximal promoter element was found to be critical for specific expression of the glucagon gene in alpha cells of the pancreas (Philippe et al., 1988). The glucagon gene is transcribed not only in the pancreas and the intestine, but also in the brain stem and hypothalamus, suggesting a neuronal-associated function (Drucker and Asa, 1988). A major site of synthesis for GHRH is the hypothalamus, where this gene expression is downregulated by pituitary hormones (Chomczynski et al., 1988). Thus, distinct regulatory elements evolved in this family of peptides, leading to tissue and cell specific expression. Factors involved in VIP gene regulation and the sites of synthesis are discussed below.

Regulation of VIP Gene Expression: Putative Control Mechanisms at the RNA Level

Phorbol esters and cAMP have been described above as having a stimulatory effect on VIP gene expression. Thus, both protein kinase A and protein kinase C are involved in the activation of the VIP gene. Moreover, calcium may also be involved in the regulation of VIP production (Waschek and Eiden, 1988). What are the physiological cues for the induction of the VIP gene? Indeed, some environmental factors that may affect VIP gene transcription have been described. Nicotine and chronic depolarization increase VIP levels (Pruss et al., 1985), whereas cholinergic agonists, serotonin, dopaminergic agonists, prostaglandins, and nerve growth factor all stimulate VIP release (Said, 1984). Eyelid fusion in primates results in an increase in VIP immunoreactivity in retinal amacrine cells (Stone et al., 1988), and opiates modulate the stress-induced increase of VIP in plasma (Crozier et al., 1988). The precise mechanism of action of the above factors is still to be elucidated.

In view of VIP's involvement in brain function, it is particularly interesting to investigate the control mechanisms regulating the production of this peptide in the brain. The VIP content of various brain areas changes markedly with development (Said, 1982, 1984; McGregor et al., 1982; Nobou et al., 1985), as measured by radioimmunoassays. A 30-fold increase in VIP is observed in the rat cerebral cortex from birth to the age of 30 d. The question is, how is this increase regulated? Since VIP-mRNA is a rare transcript (estimated at 0. 005% of the total mRNA in the rat cerebral cortex; Nishizawa et al., 1985), a highly sensitive method for its detection is required. To this aim, an RNA detection assay that uses in vitro transcribed RNA probes corresponding to the various exons of the human VIP gene was developed (Gozes and Shani, 1986). Since there is 80–90% homology between the VIP coding sequences of humans and rats (Nishizawa et al., 1985), these probes can be used to detect VIP-mRNA in both species.

mRNA prepared from rat frontal cortex was fractionated according to size by electrophoresis on agarose gels (Gozes et al., 1984 a,b). The RNA bands were blotted onto nitrocellulose filters that were used to hybridize to the specific complementary RNA fragments. When hybridization was performed using a ^{32}P-RNA probe corresponding to the VIP-encoding exon, the major hybridizing RNA band was about 2000 b (Gozes and Shani, 1986; Gozes et al., 1987d). This VIP message was similar in size to the VIP-mRNA detected in human neuroblastoma cells (Ohsawa et al., 1985) and human islet tumor cells (Tsukada et al., 1985) and was shown to contain all the exon sequences tested (Gozes at al., 1987d).

Ontogenic studies by radioimmunoassays suggest that VIP mainly appears in the postnatally-developing animal, with high concentrations in specific brain structures, such as the cerebral cortex, hypothalamus, and hippocampus (Emson et al., 1979; McGregor et al., 1982; Nobou et al., 1985). During embryonal development, significant amounts of VIP were undetectable in the brain (Emson et al., 1979; McGregor et al., 1982). In contrast, in the duodenum, as early as d 16 of gestation, limited amounts of VIP were detectable (Emson et al., 1979). This is now correlated with the detection of VIP-mRNA at this embryonic stage (Gozes et al., 1988) and the appearance of VIP responsiveness (stomach muscle relaxation) (Ito et al., 1988).

To investigate whether the developmental increase in VIP is paralleled by a change at the mRNA level, we prepared poly(A)-containing RNA from rat frontal cortex using newborn, 4-, 14-, 16-, and 30-d-old rats. The 2000-base VIP-mRNA increases about 40-fold from birth to 14 d of age; at 30 d of age, the VIP-mRNA in the cerebral cortex may decrease to about 60% of the levels at 16 d (Gozes, 1987; Gozes et al., 1987d). The content of VIP mRNA was calculated per μg total RNA, thus, because of the late glial proliferation, a decrease per μg RNA may mean no change in the neuronal population. In contrast to the cortex, the hypothalamus contained substantial amounts of VIP-mRNA already at birth when the peptide level, measured by radioimmunoassays, is very low (Gozes et al., 1987d; Nobou et al., 1985). However, by immunocytochemistry, VIP neurons and fibers were observed in the suprachiasmatic nucleus of the perinatal rat (Laemle, 1988). The reduced levels of the mature VIP-mRNA in the newborn rat cerebral cortex is in agreement with the low concentrations of the peptide (Nobou et al., 1985). The increase in the VIP-mRNA during brain maturation suggests a transcriptional regulation or mRNA stabilization. Large quantities of the VIP-mRNA are already found in the cerebral cortex at 4 d of age (Gozes, 1987), whereas VIP levels begin to increase only at about 7 d of age (McGregor et al., 1982; Nobou et al., 1985). Similar findings were obtained with other proteins during brain development (e.g., Ginzburg and Littauer, 1984), implying a more general phenomenon of regulation at the translational level. The developmental regulation of VIP gene expression may reside, in part, in specific DNA-binding proteins *(see above)*.

In aging rats, VIP concentrations were reduced by 60% in the rat lung, concomitant with a decrease in the number of VIP-immunoreactive nerve cell bodies (Geppetti et al., 1988). This decrease is not specific to the lung, thus, with aging, VIP-mRNA levels were found to reduce significantly, especially in the cerebral cortex (Gozes et al., 1988). The decrease in the cortex may be a consequence of either a reduction in the transcriptional activity of VIP neurons or death of the VIP-producing cells. The developmental and age-associated patterns of VIP gene expression may be related to its role as a neuronal survival factor (Brenneman and Eiden, 1986; *see below*).

In addition, a major site of VIP synthesis in the brain is the suprachiasmatic nucleus (SCN) of the hypothalamus (Card et al., 1988; Gozes et al., 1989a). An immunocytochemical study revealed a 36% decrease in the number of immunoreactive VIP neurons in the SCN of old rats compared to young ones, whereas the average size of the remaining VIP neurons increased in the aged rats (Chee et al., 1988), suggesting a compensatory mechanism. Moreover, manipulations, such as disruption of the optic pathway, induced a rise in VIP transcripts in the SCN (Levy Holtzman et al., 1989); while exposure to constant light significantly depressed the SCN immunoreactive VIP (Albers et al., 1987). The primary known function of the SCN appears to be its involvement in entrainment to environmental light cycles and as a circadian clock responsible for the orchestration of endogenous circadian rhythms (Van den Pol and Powley, 1979; Moore, 1983). Sensory information from the retina which influences the entrainment of cir-

cadian rhythms terminates directly in the SCN or indirectly through the intergeniculate leaflet of the thalamus (Van den Pol and Tsujimoto, 1985; Card and Moore, 1982). The diurnal cycle was found to be associated with VIP gene expression, thus VIP-mRNA concentrations were lowest in the daytime (Gozes et al., 1989d), implying that synaptic input (light) and the degree of innervation influence and modify VIP gene expression. VIP neurons in the SCN are innervated by chemically identified neurons, such as neuropeptide Y containing neurons (Hisano et al., 1988) and serotonergic nerve terminals from the raphe nuclei (Bosler and Beaudet, 1985). The VIP-containing neurons then project to other brain nuclei, including the hypothalamic (subparaventricular, preoptic, and dorsomedial) and thalamic (paraventricular and periventricular) nuclei (Van den Pol and Tsujimoto, 1985; Watts and Swanson, 1987). It has also been suggested that VIP cells may terminate on other SCN neurons that may receive additional synaptic information from neurons containing glutamic decarboxylase immunoreactivity (Van den Pol and Gorcs, 1986; Watts and Swanson, 1987). The SCN is enriched in VIP receptors (Shaffer and Moody, 1986). It is an open question as to how all these combine to regulate the expression of the VIP gene.

Synaptic connectivity may affect VIP gene expression in the SCN, as suggested above. It can also affect VIP in other neurons. Indeed, 6-hydroxy-dopamine treatment on postnatal d 2 (in rats) which delays (by 7d), sweat glands innervation by noradrenergic sympathetic axons, results in the absence of VIP immunoreactivity normally found in these axons following innervation (Stevens and Landis, 1988).

The 2000-base VIP-mRNA was not the only RNA band detected by the specific hybridization probe in the cerebral cortex (Gozes et al., 1987c); higher molecular weight, VIP-related RNA species were apparent, as well as 5000- and a 7000-base RNA bands (Gozes, 1987). Such RNA species were shown by S1 nuclease analysis to contain intron sequences (Gozes et al., 1986; Gozes et al., 1987c). The 5000-base RNA found in human neuroblastoma was also suggested to be the VIP precursor heteronuclear (hn) RNA (Ohsawa et al., 1985). The ratio of the high molecular weight, VIP-encoding RNA forms to the mature mRNA form changes during cerebral cortex development. At birth, these high molecular weight RNA forms may represent up to 80% of the total VIP-encoding RNA species, gradually reducing to about 30% at the age of 14 d and about 20% at 30 d of age. Since the VIP gene is a single copy gene (Gozes et al., 1986; Tsukada et al., 1985) that contains one promoter site (Tsukada et al., 1985), precursor RNA synthesis must precede mature RNA formation in any given cell. In a heterogeneous cell population, such as the brain, it is possible that some cells store precursor RNA whereas others process it to mature form, resulting in the multiple RNA forms observed. Thus, our results suggest that, in contrast to most genes in which the primary RNA transcript is labile and protein coding sequences are almost exclusively found in the mature mRNA form (Lewin, 1983), the primary transcript of the VIP gene is detectable in appreciable amounts (Gozes et al., 1987c). This presumptive primary transcript is detected in high quantities during early postnatal development (Gozes et al., 1987d) and perhaps during aging as well (Gozes et al., 1988).

As an initial model system for VIP gene expression, we used a human buccal tumor (Hep#3), which we have shown to contain large amounts of VIP (Gozes et al., 1984 a,b). Employing S1 nuclease analysis, we discovered that the major transcript of the VIP gene, in these tumor cells, is a nonprocessed RNA containing introns. Indeed, we obtained hybridization, and thus protection from S1 nuclease digestion, with intron sequences. In this system, the ratio of intron hybridizing RNA to the mature RNA was 8:1. Thus, the immature, unprocessed, or partially processed RNA was found in higher quantities than the mature mRNA in these tumor cells (Gozes et al.,1987c).

As for the mature mRNA, we identified a 1600-base mRNA that could also be translated into a VIP immunoreactive polypeptide (Gozes et al., 1984a,b). A 7000- and 5000-base putative precursor RNA form was also detected in the tumor. The 1600-base VIP-mRNA observed in the tumor is somewhat smaller than the major VIP-mRNA detected in the cerebral cortex. This may be trivial because of differences in the lengths of the respective poly(A) tail of the messengers. Alternatively, this could be a result of tumor-associated genomic rearrangements. To identify the exact structure of the high molecular weight RNA band and avoid any possibility of hybridization mismatches, we have undertaken the task of cloning and sequencing the corresponding cDNA. When restriction map analysis combined with sequence analysis was performed, an identity between the gene fragments and the corresponding cDNA fragments was observed (Gozes et al., 1987c).

The finding of a high molecular weight RNA hybridizing to VIP/PHM-specific sequences could be explained by several hypotheses. It is possible that some sequences of the VIP gene appear in transcripts of other genes, and hence, the high molecular weight RNA detected by Northern blot hybridization is not directly VIP-related. However, this is not likely since this band was identified with different probes corresponding to various areas of the VIP gene and cDNA; moreover, we were able to clone part of it. It is possible that the transcriptional and posttranscriptional processes are deficient in the tumor state. Alternatively, the 7000-base VIP-related RNA may be a true VIP-mRNA precursor. We would suggest that the control of VIP gene expression resides, in part, in RNA-processing mechanisms, resulting in accumulation of a high molecular weight intron containing RNA. We find elevated amounts of this precursor RNA (all cDNA clones isolated were of the precursor type) in a human tumor tissue. However, different amounts of a VIP-PHM precursor RNA can be found in various tumors (Hayakawa et al., 1984; Ohsawa et al., 1985). If the high molecular weight RNA is indeed a precursor RNA, it will be interesting to investigate what renders this RNA stable. The inability to detect precursor RNA for many other proteins might be trivial owing to the RNA preparation method and the specificity of the hybridization probes used. In addition, not all cells producing the final mature protein may contain such RNA in measurable quantities since the regulation may well be protein specific. This control process may reside in either absence or shortage of specific RNA processing enzymes; alternatively, intrinsic properties of the RNA precursor itself may render it stable to processing. Functionally, one can hypothesize that a stable RNA precursor can be stored as part of the cellular memory system to be cleaved upon induction by external stimuli, a process that will be controlled differently compared with the activation of transcription.

One definition of memory is storage of information over a period of time to be used when required. We describe here a possible mode of storage that can serve in short-term memory formation, at the cellular level (Gozes et al., 1987c). In a broader sense, it is interesting that we found a putative stable precursor for VIP-RNA, whereas Amara et al. (1982) found a high molecular weight hybridizing RNA for calcitonin, and Schwartz et al. (1984) found it for proenkephalin. Indeed, those peptides can be associated with memory-related functions requiring rapid production of neuroactive agents.

Several neuroendocrine influences have been reported for VIP gene regulation. For example, in lactating animals, many neurons in the hypothalamic paraventricular nucleus (PVN) become VIP immuno-positive (Mezey and Kiss 1985). To investigate if there is an increase of VIP synthesis in the rat hypothalamus during lactation, we have used our exon-specific RNA probes to detect and quantify VIP-mRNA in the hypothalamus. When lactating mothers were compared to

"normal" female rats, a twofold increase in the VIP-mRNA level was detected (Gozes and Shani, 1986). Thus, the VIP gene may be transcriptionally regulated by hormonal events associated with suckling and with elevated prolactin secretion. An increase in VIP-gene activity during lactation may be reflected by the finding of elevated secretion of VIP in milk (Werner et al., 1985). Employing *in situ* hybridization techniques, we were able to detect VIP-encoding transcripts in various brain cells, including the following regions: ventrolateral thalamus, neocortex, pyriform cortex, and hypothalamus, with a particularly high concentration in the suprachiasmatic nucleus (Card et al., 1988; Baldino et al., 1989). When lactating animals were compared to nonlactating animals, a twofold increase was observed in VIP transcripts in the suprachiasmatic nucleus. Since this nucleus is not directly associated with the physiology of lactation, the response of the VIP gene to lactation may be indirect. Taken together, our results suggest that lactation and the expression of the VIP gene are interrelated (Gozes et al., 1989a). Sex hormones are also associated with the control of VIP gene expression. Indeed, we found the participation of estrogen in the regulation of VIP-mRNA content in the hypothalamus (Gozes et al., 1989e). Using densitometric hybridization assays, VIP-mRNA was found to decrease significantly following ovariectomy. This decrease was largely reversed after 3 d of treatment with estradiol dibenzoate (Gozes et al., 1989e). In contrast to the female rats, no change in VIP-mRNA was observed in the male rats following orchidectomy. These results imply a sexual dimorphism regarding steroid regulation of hypothalamic VIP-gene expression. This sexual dimorphism is VIP-specific, for the amounts of somatostatin mRNA in the dorsal portion of the hypothalamic periventricular nucleus were significantly decreased following gonadectomy in both male and female rats (Werner et al., 1988; Baldino et al., 1988).

In summary, ontogenic processes, synaptic inputs, and circulating hormones all combine together to regulate the appearance and function of the VIP-mRNA and its protein products.

VIP Gene Expression: Control Mechanisms at the Protein Level

VIP gene expression can be localized, quantitated, and characterized using immunological probes, such as polyclonal or monoclonal antibodies (Gozes et al., 1983a,b; 1984a,b). To identify high molecular weight VIP immunoreactive material in tissue extracts, we separated cellular proteins by polyacrylamide gel electrophoresis, followed by electroblotting onto nitrocellulose filters and immunochemical detection (Towbin et al., 1979). Several high molecular weight VIP immunoreactive proteins were identified, suggesting either multiple precursor proteins or several VIP-related antigens. cDNA cloning from human neuroblastoma cells revealed a protein of 170 residues (Itoh et al., 1983). A possible glycosylation site, Asn-x-Thr (Pless and Lennarz, 1977), is located at amino acid residues 68–70. This suggests that the VIP/PHM precursor might be a glycoprotein (Itoh et al., 1983) that can migrate at an altered apparent molecular weight on polyacrylamide gels. Upon in vitro translation of mRNA from a human neuroblastoma producing VIP, a VIP immunoreactive protein of size corresponding to 170 amino acid residues (19,169 Dalton) was detected (Itoh et al., 1983; Obata et al., 1981). In our translation system, among the products of the Hep#3-derived mRNA, a smaller (11,000 Dalton) protein exhibited VIP immunoreactivity (Gozes et al., 1984a,b). It is possible that our translation system prematurely terminated protein synthesis directed by the particular mRNA. Interestingly, a similar molecular weight protein (11,000 Dalton) was

proposed as the VIP precursor by pulse-labeling experiments in human neuroblastoma cells (Hioki et al., 1983).

As for posttranslational processing, the *N*-terminal of human VIP is preceded by a pair of basic amino acid residues (Lys-Arg) known to be frequent sites for the posttranslational processing of hormone precursors (Itoh et al., 1983; Steiner et al., 1980; Mains et al., 1983). A glycine residue and a pair of basic amino acid residues lie adjacent to the *C*-terminal Asn residue. As with other polypeptide precursors, the glycine residue seems to serve as an amino donor for the carboxy terminal Asn-NH_2 of VIP (Itoh et al., 1983; Mains et al., 1983). The most interesting finding from the cDNA cloning was that VIP and PHM coexist on the same protein precursor. The PHM sequences are preceded by one basic amino acid, Arg, and the C-terminal methionine residue is followed by a glycine residue and a pair of basic amino acid residues (Lys-Arg); the latter is similar to VIP (Itoh et al., 1983). It should be noted that one basic amino acid is sometimes a sufficient signal for proteolytic cleavage (Mains et al., 1983). However, the different cleavage site at the *N*-terminal of VIP and PHM may be important in the regulation of posttranslational processing. Furthermore, both VIP and PHM contain a pair of internal basic amino acid residues (Lys-Lys and Arg-Lys) that could reflect a differential tissue-specific posttranslational processing pathway, yielding some "cryptic" peptides (Itoh et al., 1983). As described above, VIP-mRNA appearance during brain development precedes, by a few days, the actual appearance of VIP detected by radioimmunoassays. One possibility to be tested is that these "cryptic" peptides, which may not be detected by the antibodies used, are synthesized in the developing brain, playing a role in brain maturation processes. Molecular variants of VIP have been recently described, such as a molecule containing instead of the *C*-terminal asparagine amide of VIP, a *C*-terminal extension of Gly-Lys-Arg. This VIP variant displaces VIP in a VIP receptor assay, reacts with *N*-terminally-directed antisera in a VIP radioimmunoassay and possesses VIP-like bioactivity in an assay measuring pancreatic juice secretion in the cat (Gafvelin et al., 1988). Similarly, structurally-distinct VIP variants were obtained from rat basophilic leukemia cells; two forms were larger than VIP 1–28, whereas one form was smaller—VIP 10–28 with an asparagine-free amino acid at the carboxyl terminus rather than the amide of the VIP neuropeptide (Goetzl et al., 1988). The latter acts as a VIP antagonist (e.g,. Brenneman and Eiden, 1986). Another variant that contains the PHM sequence and the PHM-VIP spacer peptide, is peptide histidine valine 42 (PHV 42), which is associated with smooth muscle relaxation (Spokes et al., 1989). From a technical point of view, new and improved techniques are being established for VIP synthesis by artificial gene cloning (Simonosits et al., 1988), for purification (Hunter et al., 1988) and quantitation (Gjerris et al., 1988).

From an evolutionary point of view, there is a high degree of homology between the VIP-mRNA in human and rat (80–90%) and 90% homology in the VIP-coding domain (Nishizawa et al., 1985). The comparative analysis of nucleotide and amino acid sequence homologies of each domain in rat and human VIP precursors implies that selection pressures have eliminated base substitutions that might result in amino acid replacements. The high degree of VIP conservation is in agreement with its attributed important physiological roles.

Identifying VIP

Localization

In the brain, VIP has a discrete regional distribution with highest levels in the cerebral cortex, hypothalamus, amygdala, hippocampus, and corpus striatum (Said, 1982, 1986). A major hypothalamic nuclei containing VIP is the suprachiasmatic nucleus (Card et al., 1988;

and *see above*). Moreover, VIP is found within the hypothalamo–hypophysial system (Said and Porter, 1979; Shimatsu et al., 1982; Brar et al., 1985; Mikkelsen and Moller, 1988). In related structures, anterior pituitary lactotropes were found to contain VIP (Morel et al., 1982; Lam et al., 1989), which probably mediates prolactin release (Rotsztejn et al., 1980; Noguchi et al., 1988). Other brain areas containing VIP neurons are the striatum, where very small VIP-containing neurons were observed using a specific monoclonal antibody (Vincent and Reiner, 1988). *In situ* hybridization experiments, coupled with immunocytochemistry, revealed the thalamus as a major site for VIP gene expression (Gozes et al., 1989a; Baldino et al., 1989). The rat cerebral cortex has been an extensively investigated site for VIP localization. Some recent experiments by Eckenstein et al. (1988) have extended their previous studies (Eckenstein and Baughman, 1984) to show colocalization with choline acetyl transferase and VIP, and acetylcholine can be found within the same nerve endings (Agoston et al., 1988a). In addition, Hajos et al. (1988) observed bipolar and multipolar VIP neurons in the visual cortex; the latter are only a small subpopulation of the total multipolar cells. Spinal cord neurons were also shown to contain VIP in vitro (Brenneman et al., 1985) and in vivo (LaMotte, 1988). In the avian brain, coexpression of opsin and VIP-like immunoreactivity was detected in cerebrospinal fluid containing cells both in the septal and tuberal areas (Silver et al., 1988).

VIP nerve terminals also appear in the walls of cerebral pial blood vessels, probably originating from the sphenopalatine ganglion, the otic ganglion, and the carotid mini-ganglion (Suzuki et al., 1988), as well as from the superior cervical ganglion (Uemura et al., 1988; Brayden and Conway, 1988). The superior and middle cervical ganglia, as well as the stellate ganglion, contain some neurons that exhibit VIP immunoreactivity and calcitonin gene-related peptide immunoreactivity; the latter may be innervated by either somatostatin or catecholamines and *met*-enkephalin or *met*-enkephalin and neurotensin (Kummer and Heym, 1988). In humans, VIP-like immunoreactive cell bodies were detected in the sphenopalatine ganglion, and VIP nerve fibers were found innervating the lacrimal gland, suggesting that VIP is a cotransmitter for facial parasympathetic nerves that supply the eye and the lacrimal gland (Sibony et al., 1988). In this system, the pattern of innervation was identical to choline esterase distribution. Similarly, cholinergic-VIP fibers originating from the otic ganglion innervate blood vessels in the lower lip of the rat (Kaji et al., 1988). The trigeminal ganglia and the dorsal root ganglia also contain VIP immunoreactivity (Yaksh et al., 1988), as well as the vagal and glossopharyngeal afferent neurons in the rat (Helke and Hill, 1988). Coexistence of VIP and substance P-like immunoreactivity was suggested in many nerve fibers innervating the lingual salivary glands and a few intralingual ganglionic cells (Ichikawa et al., 1989).

VIP nerve terminals appear within the intestinal wall, especially at the sphincter zones in the pancreas, and the parasympathetic ganglia. Consistently high concentrations of VIP were measured in the muscle layer of the human sigmoid colon (Ferri et al., 1988). In the human gastrointestinal tract, VIP can be colocalized with neuropeptide Y (Wattchow et al., 1988). In the rat colon, one population of intramural neurons revealed the coexistence of neuropeptide Y and VIP; in addition, some perivascular calcitonin gene-related peptide-containing nerve fibers (of intrinsic origin) harbored VIP (Ekblad et al., 1988). In the inferior mesenteric ganglion of the guinea pig, analysis of 0.5 micron sections tends to confirm the coexistence of dynorphin, VIP, and cholecystokinin in fibers projecting from the colon (Masuko and Chiba, 1988). In myenteric plexus longitudinal muscle of guinea pig, VIP and acetylcholine may coexist in the same storage particles (Agoston and Whittaker, 1989).

Other nerves containing VIP are in the gall bladder, as well as urogenital organs (Klein and Burden, 1988). In the tracheobronchial tree, VIP can be localized with substance P (Dey et al., 1988). VIP can also be found in peripheral nerves, such as the sciatic and vagus nerves. Other sites of VIP localization are in the placenta, adrenal medulla, mast cells, and platelets. Recent studies have shown that multiple neuropeptides are in nerves supplying the mammalian lymph nodes, thus the distribution of VIP-immunoreactive fibers was identical to PHI-immunoreactive fibers and partially overlapped with neuropeptide Y and dopamine beta hydroxylase or substance P and calcitonin gene-related peptide. These nerves may be of heterogeneous origin: sensory, cholinergic sympathetic, and/or parasympathetic and are candidates for sensory and autonomic neuroimmunomodulation (Fink and Weihe, 1988). Organs with little or no VIP include liver and skeletal muscle. The VIP localization studies employed immunocytochemical techniques coupled with radioimmunoassays (for reviews, *see* Said, 1982, 1984, 1986; Said and Mutt, 1988).

Function

Introductory Material

The widespread distribution of VIP is correlated with its involvement in a broad spectrum of biological activities. In the chapter below, we shall discuss the VIP receptors and examine proposed mechanisms of action.

VIP Receptors

Specific receptor sites for radiolabeled VIP (Marie et al., 1985) have been demonstrated in the rat brain (Taylor and Pert, 1979) and guinea pig brain (Robberecht et al., 1978). The receptor molecule complex was identified in the intestine by covalent cross-linking (Laburthe et al., 1984) and isolated from lung (Paul and Said, 1985). Despite significant progress made on the isolation and characterization of the VIP receptor, the amino acid sequence remains unknown and it has not been cloned. Since this area has recently been reviewed elsewhere (Marchis-Mouren et al., 1988; Said and Mutt, 1988), we will comment only briefly on several recent papers. The solubilization of rat lung VIP receptors in an active state has been reported (Patthi et al., 1988). Two classes of receptors with different pharmacological properties were detected. The solubilization of active VIP receptors has also been achieved in human colonic adenocarcinoma cells (el Baattari et al., 1988). Again, two classes of receptor were observed. Cross-linking experiments on SDS gels revealed proteins of 67, 72, and 83 kD. Solubilized receptor complexes were found in two major peaks in the range of 60–70 and 270–300 kD. Depending on the receptor source, other molecular weights have been described as well (46–53 kD), and as commented above, the area has been extensively reviewed (Laburthe and Couvineau, 1988).

Mechanism of Action

The VIP receptor may very well be coupled to adenylate cyclase since a VIP-stimulated adenylate cyclase has been identified in various areas of the central nervous system (Quik et al., 1978; Deschodt-Lanckman et al., 1977), as well as in the liver and pituitary (Rostene, 1984). The ability of VIP to stimulate cyclic adenosine monophosphate (cAMP) production is somewhat more potent than norepinephrine, and the two can act synergistically (Magistretti and Schorderet, 1984). The VIP actions may be mediated via G-proteins (Fig. 3), which are signal transducers stimulating the hydrolysis of GTP to GDP (for a review on G-proteins, *see* Spiegel, 1987). Indeed, GTP and its analogs inhibit VIP-receptor binding and potentiate cAMP synthesis in response to VIP (*see* Paul, 1989). In locus ceruleus neurons, VIP causes direct excitation by inducing a Na-dependent inward current; since this effect becomes irreversible in the presence of intracellular GTP-gamma-S, mediation through a G

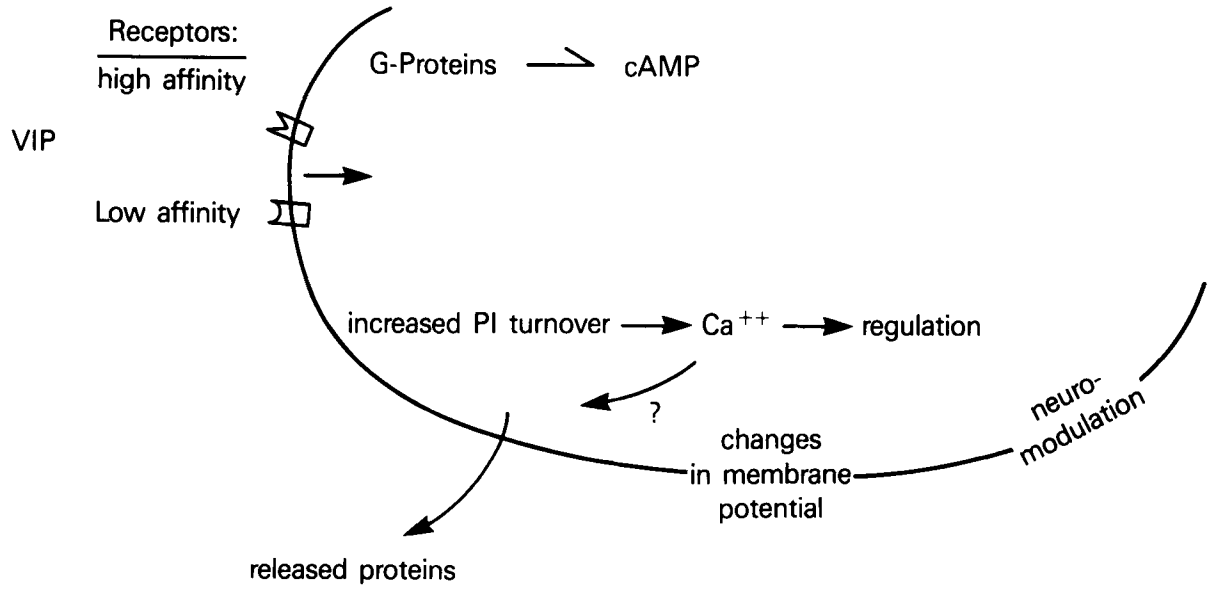

Fig. 3. VIP mechanism of action. The diagram depicts the possible pathway by which VIP exerts its function.

protein is suggested (Wang and Aghajanian, 1988). G-proteins are involved in several signal transduction pathways, such as in cAMP formation and phosphoinositides breakdown (Spiegel, 1987). If, indeed, the VIP receptor is coupled to G-proteins, the possible role of G-proteins in receptor function could explain the array of VIP effects found. It is interesting to note that high concentrations of VIP have been shown to produce increased PI turnover (Audigier et al., 1986). More recently, VIP stimulation of adrenal medulla was shown to increase the generation of inositol 1,4,5-triphosphate (IP3). This increase in IP3 has been hypothesized to stimulate the release of intracellular calcium to facilitate secretory activity in these cells (Malhotra, 1988).

Earlier studies with a wide variety of preparations indicated that treatment with VIP rapidly produced a loss of responsiveness to subsequent challenges with this peptide (*see* Rosselin et al., 1988). A short-term exposure to VIP results in an internalization of the peptide–receptor complex into clear endosomal vesicles, with a half-time in minutes (Boissard et al., 1986). VIP is degraded in lysosomes or may serve as an intracellular effector. Most VIP receptors are recycled to the cell surface (Luis et al., 1988). An ultrastructure analysis of the distribution of labeled VIP has indicated significant differences between cell types, suggesting that VIP internalization is tissue specific (Anteunis et al., 1989). Preincubation with either PHI or a protein kinase C activator can also decrease VIP binding sites and reduce VIP-stimulated adenylate cyclase, but apparently through different mechanisms (Turner et al., 1988). Direct evidence for a translocation of the VIP receptor to a light vesicle fraction has been observed after exposure to these agents. Homologous regulation of the VIP receptors has also been observed with PHM (Wiik, 1988). In an acinar cell line (AR 4-2 J), the half-life of the receptor was estimated to be 2 d, and *N*-glycosylation was necessary for translocation of the receptor (Svoboda et al., 1988). Internalization of the receptor was also blocked at 4°C. An internalized VIP receptor is dissoci-

ated from adenylate cyclase activity (Hejblum G., et al., 1988); however, the internalization process is not entirely independent of cAMP accumulation. In conclusion, it appears now that there are multiple ways for VIP signal transduction other than via elevation of cAMP.

Electrophysiology and Neuromodulation

Electrophysiological studies have shown that VIP can act directly to produce changes in membrane potential and it can work in concert with other neurotransmitters to modulate electrical responses. In early studies conducted on isolated spinal cord, VIP was found to depolarize dorsal root terminals and motoneurons (Phillis et al., 1978). Iontophoretic application of VIP to CA1 neurons of the rat hippocampal slice produced rapid depolarization and a large increase in membrane conductance (Dodd et al., 1979). VIP had a mixed response on neurons from preoptic, septal, and midbrain neurons, with some showing excitatory and others inhibitory potentials (Haskins et al., 1982). VIP increased the frequency of Ca^{2+}-dependent action potentials and prolonged individual action potentials in a pituitary cell line (Hedlund et al., 1988). These effects involved several voltage-sensitive conductances and may relate to prolactin secretion in these cells.

The stimulation of VIP receptors has been shown to increase cAMP levels in many cell types (*see above* and Rostene, 1984). However, the slow depolarization, accompanied by a decrease in membrane resistance that was observed after treatment with VIP on retinal horizontal neurons, was not elicited by analogs of cAMP or forskolin, suggesting that the changes in membrane potential were not related to the accumulation of the cyclic nucleotide (Lasater et al., 1983). This study is an example of an electrophysiological effect that is hard to reconcile with the hypothesis that VIP receptors are always linked to a cAMP-mediated process (*see above*).

VIP action in the peripheral nervous system has also been detected. In guinea pig submucosal plexus neurons (Mihara et al., 1985), slow excitatory postsynaptic potentials were produced by VIP that were reversed with membrane hyperplorization (–90 mV). Hyperpolarizing responses have been observed after treatment with VIP on a cell line derived from intestinal epithelium (Yada and Okada, 1984). In this cell, VIP, as well as other intestinal secretagogs, produced oscillations of membrane potential. The oscillatory behavior is thought to be mediated by a K^+ conductance. In addition, VIP induced rhythmic depolarization and contractions of smooth muscle from opossum esophagus (Daniel et al., 1983).

VIP has also been reported to act as a neuromodulator to noradrenergic (Ferron et al., 1985) and muscarinic responses (Mo and Dun, 1984; Kawatani et al., 1985). Regarding the cholinergic studies, VIP has a selective facilitatory effect on muscarinic excitation in sympathetic ganglia, with no apparent effect on nicotinic responses or those after electrical stimulation of the preganglionic nerve. These data indicate that VIP not only has intrinsic properties that effect electrical activity, but also interacts with other neurotransmitter systems to modulate physiological responses.

VIP and interacting neurotransmitters can either converge from two nerve terminals to act together on the target tissue or coexist with other neurotransmitters in the same terminal (*see above*). Coexistence of VIP and acetylcholine esterase (AChE) has been observed in lumbosacral sympathetic ganglia, which contain a well-defined cholinergic neuron population and are assumed to be involved in the regulation of sweat secretion (*see above* and Hokfelt et al., 1980; Haynes, 1985). Evidence suggests that VIP in cholinergic neurons may be a general characteristic of secretomotor neurons. It is now postulated that secretion and vasodilation in exocrine glands are induced by acetylcholine (ACh) and VIP, respectively, released from the same nerve terminal. In this particular system, the two putative transmitters would act on receptors located on two separate cell types. In addition, VIP

seems to potentiate ACh activity by increasing the affinity of the muscarinic receptor for ACh (Lundberg et al., 1982). In the cerebral cortex, there are two types of cholinergic innervations, one colocalized with VIP and the other not (Luine et al., 1984; Eckenstein and Baughman, 1984), suggesting interaction between VIP and ACh also in the brain.

Glial Function

Glial cells have also been shown to possess at least two classes of VIP receptors (Chneiweiss et al., 1984; Koh et al., 1984;) although there is some dispute to these findings (Staun-Olson et al., 1985). Previous radioligand binding studies have not confirmed the presence of VIP receptors on glial cells. Competition studies of VIP binding conducted in our laboratory (Gozes et al., 1989b) have suggested the presence of two classes of binding sites. The preceding studies were performed on intact astroglial cultures at reduced temperatures to slow internalization of the VIP/receptor complex. Glial cells have also been shown to respond to VIP by changes in membrane potentials. Schwann cells from the giant axon from squid respond with a hyperpolarizing action that is blocked by VIP receptor antagonists (Evans and Villegas, 1988). The physiological importance of VIP receptors on glial cells is only beginning to be recognized. Stimulation of VIP receptors on astroglia has been shown to increase glycogenolysis and produce changes in the morphology of these cells, from flat to predominantly process-bearing cells (Magistretti, 1988). Low concentrations of VIP have also been shown to interact with nonneuronal cells to produce substances necessary for neuronal survival during development (Brenneman et al., 1987b). The presence of VIP receptors on glial cells appears to vary among brain regions. Astrocytes from cortex were found to respond to VIP-stimulated cAMP formation to a greater extent than those found in the spinal cord or cerebellum (Cholewinski and Wilkin, 1988). In summary, VIP appears to be a mediator of communication between neurons and glia, a relationship of fundamental importance to neurodevelopment and function.

Pharmacology

VIP research has been hampered for many years by the lack of selective agonists and antagonists. Recently, several peptide antagonists, an analog of growth hormone releasing factor (Ac Tyr 1, D-Phe 6-GRF; Waelbroeck et al., 1985) and an analog of VIP (4-Cl-D-Phe6, Leu 7-VIP; Pandol et al., 1986) have been examined for their ability to block the physiological actions of VIP. The GRF analog was found to be effective in blocking VIP-induced hyperpolarizations in Schwann cells (Evans and Villegas, 1988) and preventing smooth muscle relaxation in opossum internal anal sphincter (Nurko et al., 1989). However, this compound did not appear to be effective in blocking feline intrapulmonary bronchial relaxation produced by VIP (Thompson et al., 1988). Importantly, in the sphincter relaxation system, the GRF analogue did not alter responses to PHI, thus suggesting specificity for this substance. The VIP analog, 4-Cl-D-Phe6, Leu 17-VIP, was reported to be somewhat more potent than the GRF analog (Evans and Villegas, 1988; Thompson et al.,1988). We have observed that both of these antagonists block VIP stimulation of cAMP accumulation in rat cortical astrocyte cultures (Gozes et al., 1989b). We have recently synthesized a hybrid VIP receptor antagonist that potently inhibits VIP binding, VIP-stimulated cAMP accumulation, and suppresses sexual activity in rats (Gozes et al., 1989b,c). This new compound is a hybrid peptide comprised of a portion of VIP and a portion of neurotensin. A synthetic peptide, L-8-K, has been shown to inhibit the specific binding of VIP to hamster pancreatic cancer cells (Singh et al., 1988). PHI also has been shown to inhibit VIP action in both a neuronal survival assay in developing cultures (Brenneman and Foster, 1987a) and in antagonizing VIP-mediated effects on sexual behavior (Gozes et al., 1989c), although PHI acts as a less potent agonist in

other VIP-responding systems. Several studies have suggested that VIP (10–28) may also have antagonistic properties in some systems (Bissonnette, et al., 1984; Brenneman and Eiden, 1986; Turner et al., 1986). Peptide T, a pentapeptide present in the external envelope protein of the human immunodeficiency virus, has been reported to block tracheal pouch relaxation mediated by VIP (Venugopalan et al., 1989). However, peptide T was not interactive with VIP receptors in the pineal (Yuwiler et al., 1989) rat intestinal epithelial cell membranes, rat liver plasma membrane, or human colonic cells (Nguyen, 1989). Although these peptides have provided utility in examining VIP-related effects, there is still no antagonist that discriminates between the high and low affinity VIP receptors that exist in many tissues.

VIP as a Secretagog

An early recognized function of VIP was its action as a secretagog. The original work on VIP-mediated release of prolactin (Ruberg et al., 1978) is now accompanied by a growing list of other substances that are secreted by VIP. As for prolactin, VIP may very well be a physiological mediator in its secretion (Rotsztejn et al., 1980; McCann et al., 1984; Mezey and Kiss, 1985; Abe et al., 1985; Fink, 1985; Kaji et al., 1985). Indeed, the concentration of VIP is several-fold higher in portal blood than peripheral blood (Said and Porter, 1979), and passive immunization with antisera against VIP resulted in inhibition of prolactin release (Abe et al., 1985; Kaji et al., 1985) and blockade of the proestrus prolactin surge (Murai et al., 1989). Recent results suggest that the prolactin producing cells of the anterior pituitary also contain VIP, which is induced by hypothyroidism (Lam et al., 1989; Reichlin, 1988), implying a direct action from close contact (Noguchi et al., 1988). Direct measurements of VIP effects on prolactin release were obtained in tissue culture using pituitary cells, such as the GH3-derived cell lines (e.g., Gourdji et al., 1979). In pituitary cells cultured in three-dimensional reaggregates, atropine, a potent muscarinic agonist, potentiated prolactin-stimulated release induced by VIP (Carmeliet and Denef, 1988). Moreover, injection of VIP to healthy women, increases prolactin levels in plasma 5 min following administration (Bataille et al., 1981; Falsetti et al., 1988). The VIP prolactin releasing activity may be associated with an activation of adenylate cyclase (Gordeladze et al., 1988). In contrast, in sheep, neither VIP nor PHI stimulate prolactin release (Thomas et al., 1988). In turkey, prolactin release induced by VIP is regulated by gonadal steroids acting directly on cells of the anterior pituitary (Knapp et al., 1988). The release of prolactin is pulsatile; in the rat it was shown that this is probably related to the concerted action of dopamine inhibition coupled to VIP stimulation (Lopez et al., 1989). Moreover, estrogen is capable of attenuating the functional coupling of the dopamine receptor and the VIP-stimulated prolactin release (Munemura et al., 1989), probably through modulation of adenylate cyclase activity (Borgudvaag and George, 1988). In the rat, an additional factor that plays a role is oxytocin; its secretion may be elevated by VIP (Bardrum et al., 1988; Samson et al., 1989) and they may act together to increase prolactin secretion (Samson et al., 1989; Arey and Freeman, 1989). However, it is proposed that VIP and serotonin are continuously active oscillatory neurotransmitters regulating oxytocin release into the portal blood. VIP acts in concert with oxytocin to stimulate the prolactin surge at 3 AM, and serotonin acts together with oxytocin to induce prolactin release at 5 PM. These daily events only eventuate in prolactin release when the mating stimulus has released the lactotroph from the inhibitory effects of dopamine (Arey and Freeman, 1989). We have found that suprachiasmatic VIP-mRNA displays a diurnal rhythm, with increased concentrations observed at nighttime (Gozes et al., 1989d; Levi-Holtzman et al., 1989). These oscillations may have a direct bearing on the periodicity of the prolactin surges.

As stated above, the earlier work on VIP-mediated release of prolactin is now accompanied by a growing list of other substances that are induced to be secreted by VIP. VIP appears to induce gonadotropin-releasing hormone from the mediobasal hypothalamus, which then releases luteinizing hormone from the pituitary (Ohtsuka et al., 1988). In isolated chromaffin cells from guinea pig adrenal, μM amounts of VIP produced a slow, calcium-dependent secretion of catecholamine (Misbahuddin et al., 1988). As determined from fura-2 studies in the chromaffin cells, this release was accompanied by only a slight increase in intracellular calcium, and no effect on Ca^{2+} uptake was observed with VIP treatment. Somatostatin release is also affected by VIP, but both increases and decreases have been reported, depending on both dose and type of preparation. In previous studies using hypothalamic slices, VIP was reported to inhibit somatostatin secretion (Epelbaum et al., 1979), whereas somatostatin secretion was increased in dispersed fetal cerebral cortical and hypothalamic cells after VIP treatment (Robbins and Landon, 1985; Tapia-Arancibia and Reichlin, 1985). A biphasic dose response to VIP has been observed in gastric secretion of somatostatin (Schmid et al., 1988). At low concentrations, VIP inhibited somatostatin release and with increasing amounts, an increase in secretion was observed. Interestingly, further studies indicated that forskolin, stimulator of adenylate cyclase, did not stimulate somatostatin release in neuronal cultures, but did in cultures containing glia (Reichlin, 1988). In contrast, VIP increased somatostatin in both neuronal and mixed cultures. In the absence of VIP, the calcium ionophore, ionomycin, and phorbol ester stimulate somatostatin release (Tapia-Arancibia et al., 1988). These data also support the involvement of a second messenger system other than cAMP. Two neurohypophyseal hormones, oxytocin and vasopressin, have been shown to be released by intracerebroventricular injections of VIP (Bardrum et al., 1988). Moreover, VIP induced a transient release of growth hormone (Bluet-Pajot et al., 1987). Other examples of proteins released by VIP include peroxidase (Dartt et al., 1988), thyroid hormone (Ahren and Hedner et al., 1989), amylase (Inoue and Kanno, 1982), and neurotrophic substances (Brenneman, 1988a). These studies serve as examples of a fundamental pharmacological action of VIP, its ability to stimulate secretion of hormones and other important substances. With this property, actions observed with VIP treatment should be carefully examined for indirect effects.

Growth and Developmental Regulator

VIP has been shown to act as a regulator of several aspects of growth and differentiation, including control of mitosis and as a releasing factor for substances that have developmental roles. VIP has been shown to have a potent, albeit small effect on astroglia proliferation in developing spinal cord cultures (Brenneman et al., 1988a). In contrast, VIP has been shown to inhibit mitosis in some other cell types. For example, in murine mesenteric lymphocytes and splenocytes, VIP inhibited the cell proliferative response to other mitogens (Ottaway and Greenberg, 1984; Stanisz et al., 1986). However, on proliferating lymphoid cells, VIP has a dual effect. In addition to the inhibition of DNA synthesis, it increases immunoglobulin A (IgA) synthesis in mesenteric lymph nodes and spleen cells and IgM synthesis in Peyer's patches (Stanisz et al., 1986). Moreover, neurological tumors with higher concentrations of VIP are associated with better differentiation and a lower metastasis rate (Allen et al., 1985). VIP is also a potent inhibitor of serum-induced DNA synthesis in cultured smooth muscle cells (Hultgardh-Nilsson et al., 1988). The inhibitory effects of VIP on cell proliferation closely relate to its ability to induce the formation of cAMP. The VIP receptors on immune and smooth muscle cells have relatively low affinity compared to those for the proliferative effects on astroglia. It is possible that these different effects on cell prolifera-

tion are attributable to different classes of VIP receptors. VIP has also been shown to inhibit growth of hamster pancreatic ductal cancer, a cell with demonstrated VIP receptors (Poston et al., 1988). The differentiation of HL-60 leukemia cells mediated by a 93 kD tyrosine protein kinase, the gene product of the c-*fes* protooncogene, was partially prevented by VIP (Yu et al., 1988). In addition, VIP has been reported to stimulate mitogenesis in hamster vas deferens cells (Mitsuhashi and Payan, 1987). VIP has also been observed to potentiate cell growth of keratinocytes with cotreatment of epidermal growth factor (Dalsgaard et al., 1989). At low concentrations of VIP, and in the presence of insulin, an increase in 3T3 cell mitosis was observed (Zurier et al., 1988). In contrast to other growth-promoting peptides, VIP did not induce an increase in cytoplasmic-free calcium or an activation of protein kinase C, but did stimulate the production of cAMP in this particular cell type. The mechanism of VIP-stimulated mitosis is not known, and regulation may vary among different cell types.

VIP has been shown to influence one important aspect of neurodevelopment. Increased survival of spinal cord neurons has been demonstrated to occur after treatment with a low concentration of VIP during a critical period of development in dissociated cultures that were electrically blocked (Brenneman and Eiden, 1986). In addition, VIP-like immunoreactivity has been found in spinal cord cultures (Brenneman and Foster, 1987a), and antisera to VIP produced significant neuronal cell death during development, suggesting the importance of this peptide, or a homologous substance, for neuronal survival (Brenneman and Eiden, 1986). The survival-promoting action of VIP was subsequently shown to be mediated indirectly through nonneuronal cells, most likely astroglia (Brenneman et al., 1987b). Both the effect of VIP on astroglial proliferation mentioned above and the action of VIP to release neurotrophic substances (Brenneman, 1988a) may mediate this developmental role for VIP. Thus, a tightly regulated VIP synthesis is probably necessary not only to maintain brain activity, but also obtain homeostasis and cellular survival.

VIP and Disease

One of the pathological syndromes described early on in VIP research involves the watery diarrhea syndrome that occurs in patients with pancreatic, bronchogenic, and other tumors (Said and Faloona, 1975). A recent progress in the treatment of diarrhea has been the development of a long-acting synthetic analog of somatostatin (SMS 201-995) that, when administered subcutaneously, has a biological half-life of 90–120 min and can be administered 2–3 times per day. Eighty-five percent of patients with pancreatic islet tumors that produce VIP responded to this drug with a reduction in the diarrhea, which often has been resistant to all other therapy (Gorden et al., 1989). It should also be noted that patients with malignant pancreatic endocrine tumors may develop symptomatic secondary hormone-induced syndromes, such as gastrointestinal perforation (Wynick et al., 1988).

VIP seems to be involved in hormone-related diseases like diabetes. An animal model for diabetes is the streptozotocin-induced diabetes in rats. Here, an increase in VIP and a decrease in calcitonin gene-related peptide was reported for the myenteric plexus of the diabetic rat ileum, which can be reversed by insulin (Burnstock et al., 1988). A similar increase in VIP was reported for the iris of the diabetic rat (Crowe and Burnstock, 1988). The increase in VIP immunoreactivity may be a transient one, leading to a later degeneration of these peptidergic nerves in the proximal colon of streptozotocin-diabetic rats (Belai et al., 1988). In addition to changes in VIP content, changes in VIP responsiveness have also been observed in the stomach fundus (Kamata et al., 1988), suggesting an involvement of VIP in the pathology of diabetes.

In the lungs, the bronchodilator component has been associated with VIP and PHM, whereas the bronchoconstrictor component

has been associated with substance P, neurokinin A, and calcitonin-gene-related peptide. These peptides are not limited to the regulation of bronchial smooth muscle tone, they may, in fact, intervene in the regulation of vascular tone, in the production of mucous and in the expression of immediate hypersensitivity reaction at the pulmonary level and, thus, may be associated with the pathophysiology of asthma (Devillier et al., 1988). Furthermore, in patients with asthma, there is a loss of detectible VIP-like immunoreactivity compared to lung tissue from patients without asthma (Ollerenshaw, 1989).

In the peripheral nervous system, VIP has been shown to be involved in the regulation of pain transmission. Thus, in the tail flick test, which is a response to thermal stimuli, VIP administration to the L5 vertebral level resulted in a decrease in the reaction time to 37% of control values at 1 min after injection (Cridland and Henry, 1988). In addition, low frequency vibration, which is a reported epidemiological cause of low-back pain, causes a two-fold increase in VIP in the L4–5 and L5–6 dorsal root ganglia and a similar decrease in substance P (Weinstein et al., 1988).

In the central nervous system, VIP may be associated with epilepsy. The E1 epileptic mouse is a model of hereditary sensory precipitated temporal lobe epilepsy. In this model system, VIP concentrations in various brain areas were found to be different than the control values and changed after seizures, suggesting that VIP plays a role in E1 mouse seizures (King and LaMotte, 1988; Kanamatsu and Hirano, 1988).

In a rat model for spontaneous hypertension, we discovered an increase in brain VIP gene expression using *in situ* hybridization histochemistry (Avidor et al., 1989). This change in VIP gene expression may be associated with the pathophysiology of the disease.

In other genetic diseases, a decreased VIP innervation of sweat glands in cystic fibrosis patients was suggested as a pathogenic factor in sweating abnormalities in disease-afflicted individuals (Heinz-Erian et al., 1985). However, the VIP gene is not the afflicted gene in this disease since it was located on human chromosome 6, which is a different site from the cystic fibrosis gene location (*see above* and Gozes et al., 1987a). Finally, at the neuroimmune axis, human autoantibodies to VIP were present in plasma from 29.6% healthy human subjects who habitually performed muscular exercise, compared to 2.3% healthy subjects who did not. These autoantibodies may not interfere with VIP-receptor binding, but are potential inhibitors of the proteolytic inactivation of VIP (Paul and Said, 1988). In addition, recent in vitro studies suggest an immunomodulatory (depressant) role for VIP. Thus, acute infusions of VIP into cannulated afferent lymphatic of popliteal lymph nodes produced prompt and marked depressions in the output of both small recirculating and blast lymphocytes into popliteal efferent lymph, with a selective effect on T4 (CD4) lymphocytes (Moore et al., 1988). Indeed, it has been suggested that the HIV (AIDS) virus may employ VIP or VIP-like receptors on brain cells and lymphocytes for intracellular access (*see below*).

Recent studies from our laboratory have indicated that low concentrations of VIP can prevent neuronal cell death produced by the external envelope protein (gp120) of the human immunodeficiency virus, the etiologic agent of Acquired Immunodeficiency Syndrome (Brenneman et al., 1988c). Previous work has suggested that VIP(1–12) is a ligand for the CD4/human immunodeficiency virus receptor (Sacerdonte et al., 1988). This hypothesis is based on the following observations: (1) the peptide T sequence of gpl20 can prevent viral infectivity in systems of low virus/target cell ratios (Pert et al., 1986); (2) peptide T shares sequence homology to VIP (7–11),TDNYT; (3) VIP and VIP fragments mimic peptide T in T4 receptor-mediated monocyte chemotaxis (Sacerdote et al., 1987; Ruff et al., 1987); (4)

Peptide T and VIP prevent neuronal cell death associated with gp120 in developing hippocampal cultures (Brenneman et al., 1988b, c); and (5) VIP is a potent inhibitor of specific peptide T receptor binding to mouse macrophage membranes and a human T-cell line (Pert et al., 1988). One interpretation of these data is that there is a high affinity VIP receptor in the CNS that has characteristics that are similar to a CD4 receptor in the brain (Taylor and Pert, 1979; Hill et al., 1986; Perry and Gordon, 1987; Pert et al., 1988). Thus, if VIP has a similar neuronal survival-promoting effect in the human brain as described in vitro (Brenneman and Eiden, 1986; Brenneman and Foster, 1987a; Brenneman, 1988a; Brenneman et al., 1987b, 1988c), then the functional interference posed by gp120 may contribute to the loss of memory-associated neuronal activity observed in AIDS dementia.

Finally, VIP has been shown to produce inhibitory effects in fear motivated behaviors (Cottrell et al., 1984). This could be mediated via modulation of the number of serotonin receptors of hippocampal neurons. Thus, the actions of VIP in the nervous system are of a complex nature encompassing receptor modulation, induction of the release of neurotrophic factors, and neurotransmission–neuromodulation.

Summary: Prospective and Perspectives

Factors that lead to a change in VIP content are now being discovered. Very sensitive techniques to identify VIP-mRNA, which were recently developed, allow further examination of VIP gene activity *in situ* at the single cell level. Such studies should contribute to the understanding of VIP gene activity in health and disease (Rostene, 1984). Recent research has shown progress from a molecular point of view, identifying control sequences on the VIP gene that interact with factors that stimulate transcription. Future research could be directed toward better understanding of tissue-specific VIP expression and answerig the question of how all the VIP-stimulating factors are acting in concert.

From a functional point of view, characterization of the VIP receptor molecules should allow for a more thorough understanding of VIP's mechanism of action. Identification of factors that are secreted by VIP will open new horizons in cellular communications. Finally, from a therapeutical point of view, designing new specific VIP agonists and antagonists may be of medical benefit for the pathological syndromes associated with VIP.

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References

Abe H., Engler D., Molitch M. E., Bollinger-Gruber J., and Reichlin S. (1985) Vasoactive intestinal peptide is a physiological mediator of prolactin release in the rat. *Endocrinology* **116,** 1383–1390.

Agoston D. V., Borroni E., and Richardson P. J. (1988a) Cholinergic surface antigen chol-1 is present in a subclass of VIP-containing rat cortical synaptosomes. *J. Neurochem.* **50,** 1659–1662.

Agoston D. V., Conlon J. M., and Whittaker V. P. (1988b) Selective depletion of the acetylcholine and vasoactive intestinal polypeptide of the guinea-pig myenteric plexus by differential mobilization of distinct transmitter pools. *Exp. Brain Res.* **72,** 535–542.

Agoston D. V. and Lisziewicz J. (1989) Calcium uptake and protein phosphorylation in myenteric neurons, like, the release of vasoactive intestinal polypeptide and acetylcholine, are frequency dependent. *J. Neurochem.* **52,** 1637–1640.

Agoston D. V. and Whittaker V. P. (1989) Characterization, by size, density, osmotic fragility, and immunoaffinity, of acetylcholine- and vasoactive intestinal polypeptide-containing storage particles from myenteric neurones of the guinea-pig. *J. Neurochem.* **52,** 1474–1480.

Ahren B. and Hedner P. (1989) Effects of VIP and helodermin on thyroid hormone secretion in the mouse. *Neuropeptides* **13,** 59–64.

Albers H. E., Ninamitani N., Stopa E., and Ferris C. F. (1987) Light selectivity alters vasoactive intestinal peptide and peptide histidine isoleucine immunoreactivity within the rat suprachiasmatic nucleus. *Brain Res.* **437,** 189–192.

Allen J., Hansen V., Maigaard S., Andersson K. E., and Forman A. (1988) Effects of some neurotransmitters and prostanoids on isolated human intracervical arteries. *Am. J. Obstet. Gynecol.* **158,** 637–641.

Allen J. M., Hoyle N. R., Yeats J. C., Ghatei M. A., Thomas D. G., and Bloom S. R. (1985) Neuropeptides in neurological tumors. *J. Neuro-Oncol.* **3,** 197–202.

Amara S. G., Jones V., Rosenfeld M. G., Ong E. S., and Evans R.M. (1982) Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. *Nature* **298,** 240–244.

Anteunis A., Astesano A., Portha B., Hejblum G., and Rosselin G. (1989) Ultrastructural analysis of VIP internalization in rat beta- and acinar cells in situ. *Am. J. Physiol.* **256,** G689–697.

Arey, B. J. and Freeman, M. E. (1989) Hypothalamic factors involved in the endogenous stimulatory rhythm regulating prolactin secretion. *Endocrinology* **124,** 878–883.

Avidor R., Eilam R., Malach R., and Gozes I. (1989) VIP-mRNA is increased in hypertensive rats. *Brain Res.*, in press.

Audigier S., Barberis C., and Jard S. (1986) Vasoactive intestinal polypeptide increases inositol phospholipid breakdown in the rat superior cervical ganglion. *Brain Res.* **376,** 363–367.

Baldino F., Jr., Fitzpatrick-McElligot S., O'Kane T. M., and Gozes I. (1988) Hormonal regulation of somatostatin messenger RNA. *Synapse* **2,** 317–325.

Baldino F., Jr., Fitzpatrick-McElligott S., Gozes I., and Card J. P. (1989) Localization of VIP and PHI-27 messenger RNA in rat thalamic and cortical neurons. *J. Molec. Neurosci.*, in press.

Barbezat G. O. and Grossman M. I. (1971) Intestinal secretion: Stimulation by peptides. *Science* **174,** 422, 423.

Bardrum B., Ottesen B., Fahrenkrug J., and Fuchs A. R. (1988) Release of oxytocin and vasopressin by intracerebroventricular vasoactive intestinal peptide. *Endocrinology* **123,** 2249–2254.

Bataille D., Talbot J. N., Milhaud G., Mutt V., and Rosselin G. (1981) Effect of vasoactive intestinal peptide (VIP) on prolactin secretion in man. *CR Seaces Acad. Sci. (III)* **292,** 511–513.

Beinfeld M. C., Korchak D. M., Roth B. L., and O'Donohue T. L. (1984) The distribution and chromatographic characterization of PHI (peptide histidine isoleucine amide) -27-like peptides in rat and porcine brain. *J. Neurosci.* **4,** 2681–2688.

Belai A., Lincoln J., Milner P., and Burnstock G. (1988) Progressive changes in adrenergic, serotonergic, and peptidergic nerves in proximal colon of streptozotocin-diabetic rats. *Gastroenterology* **9,** 1234–1241.

Bell G. I. (1986) The glucagon superfamily: precursor structure and gene organization. *Peptides* **7,** 27–36.

Bell G. I., Sanchez-Pescador R., Laybourn P. J., and Najarian R. C. (1983) Exon duplication and divergence in the human preproglucagon gene. *Nature* **304,** 368–371.

Bergman Y., Rice D., Grosschedl R., and Baltimore D. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7041–7045.

Besson J., Rotsztejn W., Labourthe M., Epelbaum J., Beaudet A., Kordon C., and Rosselin G. (1979) Vasoactive intestinal peptide (VIP): brain distribution, subcellular localization and effect of deafferentation of the hypothalamus in male rats. *Brain Research* **165,** 79–85.

Birnstiel M. L., Busslinger M., and Strub K. (1985) Transcription termination and 3' processing: The end is in site! *Cell* **41,** 349–359.

Bissonnette B. M., Collen M. J., Adachi H., Jensen R. T., and Gardner J. D. (1984) Receptors for vasoactive intestinal peptide and secretin on rat pancreatic acini. *Am. J. Physiol.* **246,** G710–G717.

Bloom S. R., Christofides N. D., Delamarter J., Buell G., Kawashima E., and Polak J.M. (1983) Diarrhoea in vipoma patients associated with cosecretion of a second active peptide (peptide hist-

idine isoleucine) explained by a single coding gene. *Lancet* **2,** 1163–1165.

Bluet-Pajot M.-T., Mounier F., Leonard J.-F., Kordon C., and Durand D. (1987) Vasoactive intestinal peptide induces a transient release of growth hormone in the rat. *Peptides* **8,** 35–38.

Bodner M., Fridkin M., and Gozes I. (1985) VIP and PHM-27 sequences are located on two adjacent exons in the human genome. *Proc. Natl. Acad. Sci. USA* **82,** 3548–3551.

Boissard C., Marie J. C., Hejblum G., Gespach C., and Rosselin G. (1986) Vasoactive intestinal peptide receptor regulation and reversible desensitization in human colonic carcinoma cells in culture. *Cancer Res.* **46,** 4406–4413.

Borgundvaag B. and George S. R. (1988) Estrogen regulation of rat anterior pituitary adenylate cyclase. *Mol. Cell Endocrinol.* **59,** 35–45.

Bosler O. and Beaudet A. (1985) VIP neurons as prime synaptic targets for serotonin afferents in rat suprachiasmatic nucleus: A combined radioautographic and immunocytochemical study. *J. Neurocytol.* **14,** 749–763.

Bowen B., Steinberg J., Laemmli U. K., and Weintroub H. (1980) The detection of DNA binding proteins by protein blotting. *Nuc. Acid Res.* **8,** 1–20.

Brar A. K., Fink G., Maletti M., and Rostene W. (1985) Vasoactive intestinal peptide in rat hypophysial portal blood: Effects of electrical stimulation of various brain areas, the oestrous cycle and anesthetics. *J. Endocrinol.* **106,** 275–280.

Brayden J. E. and Conway M. A. (1988) Neuropeptide Y and vasoactive intestinal polypeptide in cerebral arteries of the rat: Relationships between innervation pattern and mechanical response. *Regul. Pept.* **22,** 253–265.

Brenneman D. E., Eiden L. E., and Siegel R. E. (1985) Neurotrophic action of VIP on spinal cord cultures. *Peptides* **6** (suppl. 2), 35–39.

Brenneman D. E. and Eiden L. E. (1986) Vasoactive intestinal peptide and electrical activity influence neuronal survival. *Proc. Natl. Acad. Sci. USA* **83,** 1159–1162.

Brenneman D. E. and Foster G .A. (1987a) Structural specificity of peptides influencing neuronal survival during development. *Peptides* **8,** 687–694.

Brenneman D. E., Neale E. A., Foster G. A., d'Autremont S., and Westbrook G. L. (1987b) Nonneuronal cells mediate neurotrophic action of vasoactive intestinal peptide. *J. Cell Biol.* **104,** 1603–1610.

Brenneman D. E. (1988a) Two mechanisms may contribute to the neuronal survival-promoting action of VIP: Neurotrophic factor releasing activity and glial mitogenesis. *Soc. Neurosci. Abstr.* **14,** 254.

Brenneman D. E., Buzy J. M., Ruff M. R., and Pert C. B. (1988b) Peptide T sequences prevent neuronal cell death produced by the envelope protein (gpl20) of the human immunodeficiency virus. *Drug Dev. Res.* **15,** 361–369.

Brenneman D. E., Westbrook G., Fitzgerald S. P., Ennist D. L., Elkins K. L., Ruff M., and Pert C. B. (1988c) Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. *Nature* **335,** 639–642.

Burnstock G., Mirsky R., and Belai A. (1988) Reversal of nerve damage in streptozotocin-diabetic rats by acute application of insulin in vitro. *Clin. Science* **75,** 629–635.

Buscher M., Rahmsdorpf H. J., Litfin M., Karin M., and Herrlich P. (1988) Activation pathways converge to the same enhancer element. *Oncogene* **3,** 301–311.

Carati C. J., Creed K. E., and Keogh E. J. (1988) Vascular changes during penile erection in the dog. *J. Physiol.* **400,** 75–88.

Card J. P. and Moore R. Y. (1982) Ventral lateral geniculate nucleus efferents to the rat suprachiasmatic nucleus exhibit avian pancreatic polypeptide-like immunoreactivity. *Comp. Neurol.* **206,** 390–396.

Card J. P., Fitzpatrick-McElligott S., Gozes I., and Baldino F., Jr. (1988) Localization of vasopressin-, vasoactive intestinal polypeptide-, peptide histidine isoleucine- and somatostatin-mRNA in rat suprachiasmatic nucleus. *Cell Tissue Res.* **252,** 307–315.

Carmeliet P. and Denef C. (1988) Immunocytochemical and pharmacological evidence for an intrinsic cholinomimetic system modulating prolactin and growth hormone release in rat pituitary. *Endocrinology* **123,** 1128–1139.

Chee C. A., Roozendaal B., Swaab D. F., Goudsmit E., and Mirmiran M. (1988) Vasoactive intestinal polypeptide neuron changes in the senile rat suprachiasmatic nucleus. *Neurobiol. Aging* **9,** 307–312.

Chneiweiss H., Glowinski J., and Premont J. (1984) Do secretin and vasoactive intestinal peptide have independent receptors on striatal neurons and glial cells in primary cultures? *J. Neurochem.* **47,** 608–613.

Cholewinski A. J. and Wilkin G. P. (1988) Astrocytes from forebrain, cerebellum and spinal cord differ in their responses to vasoactive intestinal peptide. *J. Neurochem.* **51,** 1626–1633.

Chomczynski P., Down T. R., and Frohman L. A. (1988) Feedback regulation of growth hormone (GH)-releasing hormone gene expression by GH in rat hypothalamus. *Mol. Endocrinol.* **2,** 236–241.

Cottrell G. A., Veldhuis H. D., Rostene W. H., and de Kloet E. R. (1984) Behavioral action of vasoactive intestinal peptide (VIP). *Neuropeptides* **4,** 331–341.

Cridland R. A. and Henry J. L. (1988) Effects of intrathecal administration of neuropeptides on a spinal nociceptive reflex in the rat: VIP, galanin, CGRP, TRH, somatostatin and angiotensin II. *Neuropeptides* **11,** 23–32.

Crowe R. and Burnstock G. (1988) An increase of vasoactive intestinal polypeptide-, but not neuropeptide Y-, substance P- or catecholamine-containing nerves in the iris of the streptozotocin-induced diabetic rat. *Exp. Eye Res.* **47,** 751–759.

Crozier T. A., Drobnik L., Stafforst D., and Kettler D. (1988) Opiate modulation of the stress-induced increase of vasoactive intestinal peptide (VIP) in plasma. *Horm. Metabol. Res.* **20,** 352–356.

Dalsgaard C.-J., Hultgardh-Nilsson A., Haegerstrand A., and Nilsson J. (1989) Neuropeptides as growth factors. Possible roles in human diseases. *Regul. Pept.* **25,** 1–9.

Daniel E. E., Helmy-Elkholy A., Jager L. P., and Kannan M. S. (1983) Neither a purine nor VIP is the mediator of inhibitory nerves of opossum oesophageal smooth muscle. *J. Physiol.* (London) **336,** 243–260.

Dartt D. A., Baker A. K., Rose P. E., Murphy S. A., Ronco L. V., and Unser M. F. (1988) Role of cyclic AMP and Ca2+ in potentiation of rat lacrimal gland protein secretion. *Invest. Ophthalmol. Vis. Sci.* **29,** 1732–1738.

Deschodt-Lanckman M. Robberecht P., and Christophe J. P. (1977) Characterization of VIP-sensitive adenylate cyclase in guinea pig brain. *FEBS Lett.* **83,** 76–80.

Devillier P., Matran R., and Marsac J. (1988) The nonadrenergic, noncholinergic neuropeptide system and asthma. *Rev. Mal. Respir.* **5,** 7–20.

Dey R. D., Hoffpauir J., and Said S. I. (1988) Colocalization of vasoactive intestinal peptide- and substance P-containing nerves in cat bronchi. *Neuroscience* **24,** 275–281.

Dixson A. F., Kendrick K. M., Blank M. A., and Bloom S. R. (1984) Effects of tactile and electrical stimuli upon release of vasoactive intestinal polypeptide in the mammalian penis. *J. Endocrinol.* **100,** 249–252.

Dodd J., Kelly J. S., and Said S. I. (1979) Excitation of CAl neurones of the rat hippocampus by the octacosapeptide, vasoactive intestinal peptide (VIP). *Br. J. Pharmacol.* **66,** 126P

Drucker D. J. and Asa S. (1988) Glucagon gene expression in the vertebrate brain. *J. Biol. Chem.* **263,** 13,475–13,478.

Eckenstein F. and Baughman R.W. (1984) Two types of cholinergic innervation in cortex, one colocalized with vasoactive intestinal polypeptide. *Nature* **309,** 153–155.

Eckenstein F. P., Baughman R.W., and Quinn J. (1988) An anatomical study of cholinergic innervation in rat cerebral cortex. *Neuroscience* **25,** 457–474.

Ekblad E., Ekman R., Hákanson R., and Sundler F. (1988) Projections of peptide-containing neurons in rat colon. *Neuroscience* **27,** 655–674.

el Battari A., Martin J. M., Luis J., Pouzol O., Secchi J., Marvaldi J., and Pichon J. (1988) Solubilization of the active vasoactive intestinal peptide receptor from human colonic adenocarcinoma. *J. Biol. Chem.* **263,** 17,685–17,689.

Emson P. C., Fahrenkrug J., Schaffalitzky de Muckadel O. B., Jessell T. M., and Iversen L. L. (1978) Vasoactive intestinal polypeptide (VIP): vesicular localization and potassium evoked release from rat hypothalamus. *Brain Res.* **143,** 174–178.

Emson P. C., Gilbert R. F. T., Loren I., Fahrenkrug J., Sundler F., and Schaffalitzky de Muckadell O. B. (1979) Development of vasoactive intestinal polypeptide (VIP) containing neurones in the rat brain. *Brain Res.* **177,** 437–444.

Epelbaum J., Tapia-Arancidia L., Besson J., Rotsztejn W., and Kordon C. (1979) Vasoactive intesinal peptide inhibits release of somatostatin from hypothalamus in vitro. *Eur. J. Pharmacol.* **58,** 493–495.

Evans P. D. and Villegas J. (1988) The action of vasoactive intestinal peptide antagonists on peptidergic modulation of the squid Schwann cell. *J. Exp. Biol.* **138,** 259–269.

Fahrenkrug J., Ottesen B., and Palle C. (1988) Vasoactive intestinal polypeptide and the reproductive system. *Ann. NY Acad. Sci.* **527,** 393–404.

Falsetti L., Zanagnolo V., Gastaldi A., Memo M., and Missale C. (1988) Vasoactive intestinal polypeptide (VIP) selectively stimulates prolactin release in healthy women. *Synecol. Endocrinol.* **2,** 11–18.

Ferri G. L., Adrian T. E., Allen J. M., Soimero L., and Cancellieri A. (1988) Intramural distribution of regulatory peptides in the sigmoid-recto-anal region of the human gut. *Gut* **29,** 762–768.

Ferron A., Siggins G. R., and Bloom F. E. (1985) Vasoactive Intestinal Peptide acts synergistically with norepinephrine to depress spontaneous discharge rate in cerebral cortical neurons. *Proc. Natl. Acad. Sci. USA* **82,** 8810–8812.

Fink G. (1985) Has the prolactin inhibiting peptide at last been found? *Nature* **316,** 487, 488.

Fink J. S., Verhave M., Kasper S., Tsukada T., Mandel G., and Goodman R. H. (1988) The CGTCA sequence motif is essential for biological activity of the vasoactive intestinal peptide gene cAMP-regulated enhancer. *Proc. Natl. Acad. Sci. USA* **85,** 6662–6666.

Fink T. and Weihe E. (1988) Multiple neuropeptides in nerves supplying mammalian lymph nodes: Messenger candidates for sensory and autonomic neurotransmission. *Neurosci. Lett.* **90,** 39–44.

Gafvelin G., Andersson M., Dimaline R., Jornvall H., and Mutt V. (1988) Isolation and characterization of a variant form of vasoactive intestinal polypeptide. *Peptides* **9,** 469–474.

Geppetti P., De Rossi M., Mione M. C., Renzi D., and Amenta F. (1988) Age-related changes in vasoactive intestinal polypeptide levels and distribution in the rat lung. *J. Neural Transm.* **74,** 1–10.

Giachetti A., Said S. I., Reynolds R. C., and Koniges F. C. (1977) Vasoactive intestinal polypeptide in brain: Localization and release from isolated nerve terminals. *Proc. Natl. Acad. Sci. USA* **74,** 3424–3428.

Ginzburg I. and Littauer U. Z. (1984) The expression and cellular organization of microtubule proteins: Brain specific probes in the study of differential expression during development. *Molecular Biology of the Cytoskeleton* (Borisy G. G., Cleveland D. W., and Murphy D. B., eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp. 357–366.

Gjerris A., Gjerris F., Sorensen P. S., Sorensen E. B. and Christensen N. J. (1988) Do concentrations of neurotransmitters measured in lumbar cerebrospinal fluid reflect the concentrations at brain level. *Acta Neurochir.* **91,** 55–59.

Goetzl E. J., Sreedharan S. P., and Turck C. W. (1988) Structurally distinctive vasoactive intestinal peptides from rat basophilic leukemia cells. *J. Biol. Chem.* **263,** 9083–9086.

Gordeladze J. O., Sletholt K., Thorn N. A., and Gautvik K. M. (1988) Hormone-sensitive adenylate cyclase of prolactin-producing rat pituitary adenoma (GH4Cl) cells: Molecular organization. *Eur. J. Biochem.* **177,** 665–672.

Gorden P., Comi R. J., and Maton P. N. (1989) NIH conference. Somatostatin and somatostatin analogue (SMS 201-995) in treatment of hormone-secreting tumors of the pituitary and gastrointestinal tract and non-neoplastic diseases of the gut. *Ann. Intern. Med.* **110,** 35–50.

Gotoh E., Yamagami T., Yamamoto H., and Okamoto, H. (1988) Chromosomal assignment of human VIP/PHM-27 gene to 6q26–q27 region by spot blot hybridization and in situ hybridization. *Biochem. Int.* **17,** 555–562.

Gourdji D., Bataille D., Vauclin N., Grouselle D., Rosselin G., and Tixier-Vidal A. (1979) Vasoactive intestinal peptide (VIP) stimulates prolactin (PRL) release and cAMP production in a rat pituitary cell line (GH3/B6). Additive effects of VIP and TRH on PRL release. *FEBS Lett.* **104,** 165–168.

Gozes I., Milner R. J., Liu F.-T., Johnson E., Battenberg E. L. F., Katz D., and Bloom F. E. (1983a) Monoclonal antibodies against vasoactive intestinal polypeptide: Studies of structure and related antigens. *J. Neurochem.* **41,** 549–556.

Gozes I., O'Connor D. T., and Bloom F. E. (1983b) A possible high molecular weight precursor to vasoactive intestinal polypeptide sequestered into catecholamine storage granule preparation. *Regul. Pept.* **6,** 111–119.

Gozes I., Bodner M., Shani Y., and Fridkin M. (1984a) Detection of mRNAs containing regulatory peptide sequences using synthetic oligodeoxynucleotide probes. *J. Cell Biochem.* **26,** 147–156.

Gozes I., Bodner M., Shwartz H., Shani Y., and Fridkin M. (1984b) Studies toward the biosynhesis of vasoactive intestinal peptide (VIP). *Peptides* **5,** 161–166.

Gozes I., Bodner M., Shani Y., and Fridkin M. (1986) Structure and expression of the vasoactive intes-

tinal peptide (VIP) gene in a human tumor. *Peptides* **7,** 1–6.

Gozes I. and Shani Y. (1986) Hypothalamic VIP-mRNA is increased in lactating rats. *Endocrinol.* **119,** 2497–2501.

Gozes I. and Tsafriri A. (1986) Detection of VIP-encoding mRNA in the rat ovaries. *Endocrinol.* **119,** 2606–2610.

Gozes I. (1987) VIP gene expression. *Brain Peptides* (update volume 1), chapter 10 (Martin J. B., Brownstein M. J., and Krieger D., eds.), Wiley, NY, pp. 141–162.

Gozes I., Avidor R., Katznelson D., Yahav Y., Croce C., and Hubner K. (1987a) The gene encoding vasoactive intestinal peptide is located on human chromosome 6p21–6qter. *Hum. Genet.* **75,** 41–44.

Gozes I., Nakai H., Byers M., Avidor R., Weinstein Y., Shani Y., and Shows T. B. (1987b) Sequential expression in the nervous system of the VIP and c-myb genes located on the human chromosomal region 6q24. *Somatic Cell and Mol. Gen.* **13,** 305–313.

Gozes I., Giladi E., and Shani Y. (1987c) VIP-gene expression: Putative mechanism of information storage at the RNA level. *J. Neurochem.* **48,** 1136–1141.

Gozes I., Shani Y., and Rostene W. H. (1987d) Developmental expression of the VIP-gene in brain and intestine. *Mol. Brain Res.* **2,** 137–148.

Gozes I., Shachter P., Shani Y., and Giladi E. (1988) Vasoactive intestinal peptide gene expression from embryos to aging rats. *Neuroendocrinology* **47,** 27–31.

Gozes I., Avidor R., Biegon A., and Baldino F., Jr. (1989a) Lactation elevates vasoactive intestinal peptide messenger ribonucleic acid in rat suprachiasmatic nucleus. *Endocrinol.* **124,** 181–186.

Gozes I., Fridkin M., and Brenneman D. E. (1989b) A novel antagonist to vasoactive intestinal peptide. *Soc. Neurosci. Abstr.* **15,** 216 (abstr. 921).

Gozes I., Meltzer E., Rubinrout S., Brenneman D. E., and Fridkin M. (1989c) Vasoactive intestinal peptide potentiates sexual behavior: Inhibition by novel antagonist. *Endocrinol.*, in press.

Gozes I., Shani Y., Liu B., and Burbach J. P. H. (1989d) Diurnal variations in vasoactive intestinal peptide messenger RNA in the suprachiasmatic nucleus of the rat. *Neurosci. Res. Commun.*, in press.

Gozes I., Werner H., Fawzi M. A. A., Shani Y., Fridkin M., and Koch Y. (1989e) Estrogen regulation of vasoactive intestinal peptide mRNA in the rat hypothalamus. *J. Mol. Neurosci.* **1,** 55–61.

Gubits R. M., Hazelton J. L., and Simantov R. (1988) Variation in c-fos gene expression during rat brain development. *Mol. Brain Res.* **3,** 197–202.

Guillemin R., Brazeau P., Bohlen P., Esch F., Fing N., and Wehrenberg W. B. (1982) Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. *Science* **218,** 505–507.

Hajos F., Zilles K., Gallatz K., Schleicher A., and Kaplan I. (1988) Ramification patterns of vasoactive intestinal polypeptide (VIP)-cells in the rat primary visual cortex. An immunohistochemical study. *Anat. Embryol.* **178,** 197–206.

Haskins J. T., Samson W. K., and Moss R. L. (1982) Evidence for vasoactive intestinal polypeptide (VIP) altering the firing rate of preoptic, septal and midbrain central gray neurons. *Reg. Pept.* **3,** 113–123.

Hayakawa Y., Obata K.-I., Itoh N., Yanaihara N., and Okamoto H. (1984) Cyclic AMP regulation of pro-vasoactive intestinal polypeptide/PHM-27 synthesis in human neuroblastoma cells. *J. Biol. Chem.* **259,** 9207–9211.

Haynes L. W. (1985) Modulation of cholinergic transmission by VIP, a peptide co-transmitter. *Trends Pharmacol.* **6,** 427, 428.

Hedlund B., Dufy B., and Barker L. (1988) Vasoactive intestinal polypeptide alters GH3/B6 pituitary cell excitability. *Pflugers Arch.* **411,** 173–179.

Heinrich G., Gros P., and Habener J. F. (1984) Glucagon gene sequence: Four of six exons encode separate functional domains of rat pre-proglucagon. *J. Biol. Chem.* **259,** 14,082–14,087.

Heinz-Erian P., Dey R. D., Flux M., and Said S. I. (1985) Deficient vasoactive intestinal peptide innervation in the sweat glands of cystic fibrosis patients. *Science* **229,** 1407, 1408.

Hejblum G., Gali P., Boissard C., Astesamo A., Hui B., and Rosselin G. (1988) Combined ultrastructural and biochemical study of cellular processing of vasoactive intestinal peptide and its receptors in human colonic carcinoma cells in culture. *Cancer Res.* **48,** 6201–6210.

Helke C. J. and Hill K. M. (1988) Immunohistochemical study of neuropeptides in vagal and glossopharyngeal afferent neurons in the rat. *Neuroscience* **26,** 539–551.

Hill J. M., Farrar W. L., and Pert C. B. (1986) Localization of the T4 antigen/AIDS virus receptor in

monkey and rat brain: Prominence in cortical regions. *Psychopharmacol. Bull.* **22,** 689–694.

Hioki Y., Hoshino M., Yanaihara C., Ogino K., Sato H., and Yanaihara N. (1983) Characterization of VIP-like immunoreactivity in a human neuroblastoma cell line, *Peptide Chemistry 1982,* Sakakibara S., ed., Protein Research Foundation, Osaka, Japan, pp. 263–266.

Hisano S., Chikamori-Aoyama M., Katoh S., Kagotani Y., Daikoku S., and Chihara K. (1988) Suprachiasmatic nucleus neurons immunoreactive for vasoactive intestinal neuropeptide have synaptic contacts with axons immunoreactive for neuropeptide Y: An immunoelectron microscopic study in the rat. *Neurosci. Lett.* **26,** 145–150.

Hokfelt T., Johansson O., Ljungdahl A., Lunberg J. M., and Schultzberg M. (1980) Peptidergic neurons. *Nature* **284,** 515–521.

Hultgardh-Nilsson A., Nilsson J., Jonzon B., and Dalsgaard C. J. (1988) Growth-inhibitory properties of vasoactive intestinal peptide. *Reg. Pept.* **22,** 267–274.

Hunter L. W., Rorie D. K., Yaksh T. L., and Tyce G. M. (1988) Concurrent separation of catecholamines, vasoactive intestinal peptide, and neuropeptide Y in superfusate and tissue extract. *Anal. Biochem.* **173,** 340–352.

Hyman S. E., Comb M., Lin Y.-S., Pearlberg J., Green M. R., and Goodman H. M. (1988) A common transacting factor is involved in transcriptional regulation of neurotransmitter genes by cyclic AMP. *Mol. Cell. Biol.* **8,** 4225–4233.

Ichikawa H., Nishikawa S., Wakisaka S., Matsuo S., and Takano Y. (1988) Coexistence of vasoactive intestinal polypeptide- and substance P-like immunoreactivities in the tongue of the guinea pig. *Neurosci. Lett.* **89,** 283–285.

Inoue Y. and Kanno T. (1982) Secretory effects of vasoactive intestinal polypeptide (VIP), adrenaline and carbachol in isolated lobules of the rat parotid gland. *Bio. Res.* **3,** 384–389.

Ito S., Kimura A., and Ohga A. (1988) Development of noncholinergic, non-adrenergic excitatory and inhibitory responses to intramural nerve stimulation in rat stomach. *Br. J. Pharmacol.* **93,** 684–692.

Itoh N., Obata K., Yanaihara N., and Okamoto H. (1983) Human preprovasoactive intestinal polypeptide contains a novel PHI-27-Like peptide, PHM-27. *Nature* **304,** 547–549.

Johansson O. and Lundberg J. M. (1981) Ultrastructural localization of VIP-Like immunoreactivity in large dense-core resides of 'cholinergic-type' nerve terminals in cat exocrine glands. *Neuroscience* **6,** 847–862.

Kaji A., Shigematsu H., Fujita K., Maeda T., and Watanabe S. (1988) Parasympathetic innervation of cutaneous blood vessels by vasoactive intestinal polypeptide-immunoreactive and acetylcholinesterase-positive nerves: Histochemical and experimental study on rat lower lip. *Neurosci.* **25,** 353–362.

Kaji H., Kazuo C., Abe H., Kita T., Kashio Y., Okimura Y., and Fujita T. (1985) Effect of passive immunization with antisera to vasoactive intestinal polypeptide and peptide histidine isoleucine amide on S-Hydroxy-L-Tryptophan-induced prolactin release in rats. *Endocrinol.* **117,** 1914–1919.

Kamata K., Sakamoto A., and Kasuya Y. (1988) Changes in sensitivity of the rat stomach fundus to various drugs in streptozotocin-induced diabetic rats. *Jpn. J. Pharmacol.* **47,** 99–102.

Kanamatsu T. and Hirano S. (1988) Differences in ME-LI and VIP- LI in discrete brain regions of seizure-naive and seizure-experienced E1 mice *Neurochem. Res.* **13,** 983–988.

Kawatani M., Rutigliano M., and de Groat W. C. (1985) Depolarization and muscarinic excitation induced in a sympathetic ganglion by vasoactive intestinal polypeptide. *Science* **229,** 879–881.

Kerins C. and Said S. I. (1973) Hyperglycemic and glycogenolotic effects of vasoactive intestinal polypeptide. *Proc. Soc. Exp. Biol. Med.* **142,** 1014–1017.

King J. T., Jr. and LaMotte C. C. (1988) VIP-, SS-, and GABA-like immunoreactivity in the mid-hippocampal region of E1 (epileptic) and C578L/6 mice. *Brain Res.* **475,** 192–197.

Klein C. M. and Burden H. W. (1988) Substance P- and vasoactive intestinal polypeptide (VIP)-immunoreactive nerve fibers in relation to ovarian postganglionic perikarya in para- and prevertebral ganglia: Evidence from combined retrograde tracing and immunocytochemistry. *Cell Tissue Res.* **252,** 403–410.

Knapp T. R., Fehrer S. C., Silsby J. L., Porter T. E., and Behnke E. J. (1988) Gonadal steroid modulation of basal and vasoactive intestinal polypeptide-stimulated prolactin release by turkey

anterior pituitary cells. *Gen. Comp. Endocrinol.* **72,** 226–236.

Koh S.-W. M., Kyritsis A., and Chader G. J. (1984) Interaction of neuropeptides and cultured glial (Muller) cells of the chick retina: Elevation of intracellular cyclic AMP by vasoactive intestinal peptide and glucagon. *J. Neurochem.* **43,** 199–203.

Krieger D. T., Brownstein M. J., and Martin J. B. (1983) *Brain Peptides,* Wiley, New York.

Kummer W. and Heym C. (1988) Neuropeptide distribution in the cervico-thoracic paravertebral ganglia of the cat with particular reference to calcitonin gene-related peptide immunoreactivity. *Cell Tissue Res.* **252,** 463–471.

Laburthe M. and Couvinneau A. (1988) Molecular analysis of vasoactive intestinal peptide receptors. Vasoactive intestinal peptide and related peptides, Said S. I. and Mutt V., eds., *Ann. NY Acad Sci.* **527,** 296–313.

Laburthe M., Breant B., and Rouyer-Fessard C. (1984) Molecular identification of receptors for vasoactive intestinal peptide in rat intestinal epithelium by covalent cross-linking. Evidence for two classes of binding sites with different structural and functional properties. *Eur. J. Biochem.* **139,** 181–187.

Laemle L. K. (1988) Vasoactive intestinal polypeptide (VIP)-like immunoreactivity in the suprachiasmatic nucleus of the perinatal rat. *Brain Res.* **469,** 308–312.

Lam K. S., Lechan R. M., Minamitani N., Segerson T. P., and Reichlin S. (1989) Vasoactive intestinal peptide in the anterior pituitary is increased in hypothyroidism. *Endocrinol.* **124,** 1077–1084.

LaMotte C. C. (1988) Lamina X of primate spinal cord: distribution of five neuropeptides and serotonin. *Neurosci.* **25,** 639–658.

Lasater E. M., Watling K. J., and Dowling J. E. (1983) Vasoactive intestinal peptide alters membrane potential and cyclic nucleotide levels in retinal horizontal cells. *Science* **221,** 1070–1072.

Lee T. J., Saito A., and Berezin I. (1984) Vasoactive intestinal polypeptide-like substance: the potential transmitter for cerebral vasodilation. *Science* **224,** 898–901.

Levy Holtzman R., Malach R., and Gozes I. (1989) Disruption of the optic pathway during development affects vasoactive intestinal peptide mRNA expression. *New Biologist,* in press.

Lewin B. (1983) *Genes,* Wiley, New York.

Lin Y.-S. and Green M. R. (1988) Interaction of a common cellular transcription factor, ATF, with regulatory elements in both Ela- and cyclic AMP-inducible promoters. *Proc. Natl. Acad. Sci. USA* **85,** 3396–3400.

Linder S. T., Barkhem A., Norberg H., Person M., Schalling M., Hokfelt T., and Magnusson G. (1987) Structure and expression of the gene encoding the vasoactive intestinal peptide precursor. *Proc. Natl. Acad. Sci. USA* **84,** 605–609.

Liu Y.-X., Kasson B. G., Dahl K. D., and Hsueh A. J. W. (1987) Vasoactive intestinal peptide stimulate plasminogen activator activity by cultured rat granulosa cells and cumulus-oocyte complexes. *Peptide* **8,** 29–33.

Lopez F. J., Dominguez J. R., Sanchez-Franco F., and Negro Vilar A. (1989) Role of dopamine and vasoactive intestinal peptide in the control of pulsatile prolactin secretion. *Endocrinol.* **124,** 527–535.

Luine V. N., Rostene W., Rhodes J., and McEwen B. S. (1984) Activation of choline acetyl transferase by vasoactive intestinal peptide. *J. Neurochem.* **42,** 1131–1134.

Luis J., Martin J. M., el Battari A., Marvaldi J., and Pichon J. (1988) The vasoactive intestinal peptide (VIP) receptor: recent data and hypothesis. *Biochimie* **70,** 1311–1322.

Lundberg J. M., Hedlund B., and Bartfai T. (1982) Vasoactive intestinal polypeptide enhances muscarinic ligand binding in cat submandibular salivary gland. *Nature* **295,** 147–149.

Magistretti P. J., Morrison J. H., Shoemaker W. J., Sapin V., and Bloom F. E. (1981) Vasoactive intestinal polypeptide induces glycogenolysis in mouse cortical slices: A possible regulatory mechanism for the local control of energy metabolism. *Proc. Natl. Acad. Sci. USA* **78,** 6535– 6539.

Magistretti P. J. and Schorderet M. (1984) VIP and noradrenaline act synergistically to increase cyclic AMP in cerebral cortex. *Nature* **308,** 280–282.

Magistretti P. J. (1988) Regulation of glycogenolysis by neurotransmitters in the central nervous system. *Diabetes Metab.* **14,** 237–246.

Mains R. E., Eipper B. A., Glembotski C. C., and Dores R. M. (1983) Strategies for the biosynthesis of bioactive peptides. *Trends Neurosci.* **6,** 229–235.

Makhlouf G. M., Zfass A. M., Said S. I., and Schebalin M. (1978) Effects of synthetic vasoactive intestinal peptide (VIP), secretin and their partial sequences or gastric secretion. *Proc. Soc. Exp. Biol. Med.* **157,** 565–568.

Malhotra R. K., Wakade T. D., and Wakade A. R. (1988) Vasoactive intestinal polypeptide and muscarine mobilize intracellular Ca2+ through breakdown of phosphoinositides to induce catecholamine secretion. *J. Biol. Chem.* **263,** 2123–2126.

Marchis-Mouren G., Martin J. M., Luis J., el Battari A., Muller J. M., Marvaldi J., and Pichon J. (1988) HT 29, a model cell line: Stimulation by the vasoactive intestinal peptide (VIP); VIP receptor structure and metabolism. *Biochimie* **70,** 663–667.

Marie J.-C., Hoa D., Jackson R., Hejblum G., and Rosselin G. (1985) The biological relevance of HPLC-purified vasoactive intestinal polypeptide monoiodinated at tyrosine 10 or tyrosine 22. *Reg. Pept.* **12,** 113–123.

Masuko S. and Chiba T. (1988) Projection pathways, co-existence of peptides and synaptic organization of nerve fibers in the inferior mesenteric ganglion of the guinea-pig. *Cell Tissue Res.* **253,** 507–516.

Mayo K. E., Cerelli G. M., Lebo R. V., Bruce B .D., Rosenfeld M. G., and Evans R. M. (1985) Gene encoding human growth hormone-releasing factor precursor: Structure, sequence, and chromosomal assignment. *Proc. Natl. Acad. Sci. USA* **82,** 63–67.

McCann S. M., Lumpkin M. D., Mizunuma H., Khorram O., Ottlecz A., and Samson W. K. (1984) Peptidergic and dopaminergic control of prolactin release. *Trends Neurosci.* **7,** 127–131.

McGregor G. P., Woodhams P. L., O'Shaughnessy D. J., Ghatei M. A., Polak J. M., and Bloom S. R. (1982) Developmental changes in bombesin, substance P, somatostatin and vasoactive intestinal polypeptide in the rat brain. *Neurosci. Lett.* **28,** 21.

Mezey E. and Kiss J. Z. (1985) Vasoactive intestinal peptide-containing neurons in the paraventricular nucleus may participate in regulating prolactin secretion. *Proc. Natl. Acad. Sci. USA* **82,** 245–247.

Mihara S., Katayama Y., and Nishi S. (1985) Slow postsynaptic potentials in neurons of submucous plexus of guinea-pig caecum and their mimicry by noradrenaline and various peptides. *Neurosci.* **16,** 1057–1068.

Mikkelsen J. D. and Moller M. (1988) Vasoactive intestinal peptide in the hypothalamo–hypophysial system of the Mongolian gerbil. *J. Comp. Neurol.* **1,** 87–98.

Misbahuddin M., Oka M., Nakanishi A., and Morita K. (1988) Stimulatory effect of vasoactive intestinal polypeptide on catecholamine secretion from isolated guinea pig adrenal chromaffin cells. *Neurosci. Lett.* **92,** 202–206.

Miskimins W. K., Roberts M. P., McClelland A., and Ruddle F. H. (1985) Use of a protein-blotting procedure to identify nuclear proteins that recognize the promoter region of the transferrin receptor gene. *Proc. Natl. Acad. Sci. USA* **82,** 6741–6744.

Mitsuhashi M. and Payan D. G. (1987) The mitogenic effects of vasoactive neuropeptides on cultured smooth muscle cell lines. *Life Sci.* **40,** 853– 861.

Mo N. and Dun N. J. (1984) Vasoactive intestinal polypeptide facilitates muscarinic transmission in mammalian sympathetic ganglia. *Neurosci. Lett.* **52,** 19–23.

Montminy M. R. and Bilezikjian L. M. (1987) Binding of a nuclear protein to the cyclic AMP responsive element of the somatostatin gene. *Nature* **328,** 175–178.

Moore D. D., Marks A. R., Buckley D. I., Kapler G., Payvar F., and Goodman H. M. (1985) The first intron of the human growth hormone gene contains a binding site for glucocorticoid receptor. *Proc. Natl. Acad Sci USA* **82,** 699–702.

Moore T. C., Spruck C. H., and Said S. I. (1988) Depression of lymphocyte traffic in sheep by vasoactive intestinal peptide (VIP). *Immunol.* **64,** 475–478.

Moore R. Y. (1983) Organization and function of a central nervous system circadian oscillator: The suprachiasmatic hypothalamic nucleus. *Fed. Proc.* **42,** 2783–2789.

Morel G., Besson J., Rosselin G., and Dubois P. M. (1982) Ultrastructural evidence for endogenous vasoactive intestinal peptide-like immunoreactivity in the pituitary gland. *Neuroendocrinol.* **34,** 85–89.

Morrison J. H., Magistretti P. J., Benoit R., and Bloom F. E. (1984) The distribution and morphological characteristics of the intracortical VIP-positive cells: An immunohistochemical analysis. *Brain Res.* **292,** 269–282.

Munemura M., Agui T., and Sibley D. R. (1989) Chronic estrogen treatment promotes a functional uncoupling of the D2 dopamine receptor in rat anterior pituitary gland. *Endocrinol.* **124,** 346–355.

Murai I., Reichlin S., and Ben-Jonathan N. (1989) The peak phase of the proestrous prolactin surge is blocked by either posterior pituitary lobectomy or antisera to vasoactive intestinal peptide. *Endocrinol.* **124,** 1050–1055.

Nguyen T. D. (1989) Peptide T from human immunodeficiency virus envelope does not interact with hepatic, intestinal and colonic vasoactive intestinal peptide (VIP) receptors. *Peptides* **9,** 425–428.

Nichols R., Lee T. D., and Andrews P. C. (1988) Pancreatic proglucagon processing: Isolation and structures of glucagon and glucagon-like peptide from gene I. *Endocrinol.* **123,** 2639–2645.

Nishizawa M., Hayakawa Y., Yanihara N., and Okamoto H. (1985) Nucleotide sequence divergence and functional constraint in VIP precursor mRNA evolution between human and rat. *FEBS Lett.* **183,** 55–59.

Nobou F., Besson J., Rostene W., and Rosselin G. (1985) Ontogeny of vasoactive intestinal peptide and somatostatin in different structures of the rat brain: Effects of hypo- and hypercorticism. *Dev. Brain Res.* **20,** 296–301.

Noguchi T., Sugisaki T., Kanamatsu T., Satoh I., and Nishikawa, N. (1988) Reduced vasoactive intestinal polypeptide immunoreactivity in the pituitaries of hormone-deficient mutant mice. *J. Endocrinol.* **118,** 179–185.

Nurko S., Dunn B. M., and Rattan S. (1989) Peptide histidine isoleucine and vasoactive intestinal polypeptide cause relaxation of opossum internal anal sphincter via two distinct receptors. *Gastroenterol.* **96,** 403–414.

Obata K., Itoh N., Okamoto H., Yanaihara C., Yanaihara N., and Suzuki T. (1981) Identification and processing of biosynthetic precursors to vasoactive intestinal polypeptide in human neuroblastoma cells. *FEBS Lett.* **136,** 123–126.

Ohsawa K., Hayakawa Y., Nishiawa M., Yamagami T., Yamamoto H., Yanaihara N., and Okamoto H. (1985) Synergistic stimulation of VIP/PHM-27 gene expression by cyclic AMP and phorbol esters in human neuroblastoma cells. *Biochem. Biophys. Res. Commun.* **132,** 885–891.

Ohtsuka S., Miyake A., Nishizaki T., Tasaka K., and Tanizawa O. (1988) Vasoactive intestinal peptide stimulates gonadotrophic-releasing hormone release from rat hypothalamus in vitro. *Acta Endocrinol.* **117,** 399–402.

Ollerenshaw S., Jarvis D., Woolcock A., Sullivan C., and Scheibner T. (1989) Absence of immunoreactive vasoactive intestinal peptide in tissue from the lungs of patients with asthma. *N. Engl. J. Med.* **320,** 1244–1248.

Ottaway C. A. and Greenberg G. R. (1984) Interaction of vasoactive intestinal peptide with mouse lymphocytes: Specific binding and the modulation of mitogen responses. *J. Immunol.* **132,** 417–423.

Ottesen B., Wagner G., Virag R., and Fahrenkrug J. (1984) Penile erection: Possible role for vasoactive intestinal polypeptide as a neurotransmitter. *Br. Med. J.* **288,** 9–11.

Pandol S. J., Dharmsathaphorn K., Schoeffield M. S., Vale W., and Rivier J. (1986) Vasoactive intestinal peptide receptor antagonist (4Cl-D-Phe6, Leu17) VIP. *Am. J. Physiol.* **250,** G553–G557.

Patthi S., Simerson S., and Velicelebi G. (1988) Solubilization of rat lung vasoactive intestinal peptide receptors in the active state. Characterization of the binding properties and comparison with membrane-bound receptors. *J. Biol. Chem.* **263,** 19,363–19,369.

Paul S. (1989) Decreased selectivity of vasoactive intestinal peptide receptors by GTP. *Biochem. Pharmacol.* **38,** 699–702.

Paul S. and Said S. I. (1985) Solubilization and physical separation of active high- and low-affinity receptors for vasoactive intestinal peptide (VIP). *Reg. Pept.* **3,** S52.

Paul S. and Said S. I. (1988) Human autoantibody to vasoactive intestinal peptide: Increased incidence in muscular exercise. *Life Science* **43,** 1079–1084.

Pelletier G., Leclerc R., Puviani R., and Polak J. (1981) Electron immunocytochemistry in vasoactive intestinal polypeptide (VIP) in the rat brain. *Brain Research* **210,** 356–360.

Perry V. H. and Gordon S. J. (1987) Modulation of CD4 antigen on macrophages and microglia in rat brain. *J. Exp. Med.* **166,** 1138–1143.

Pert C. B., Hill J. M., Ruff M. R., Berman R. M., Robey W. G., Arthur L. O., Ruscetti F. W., and Farrar W. L. (1986) Octapeptides deduced from the neuropeptide receptor-like pattern of antigen T4 in brain potently inhibit human immunodeficiency virus receptor binding and T-cell infectivity. *Proc. Natl. Acad. Sci. USA* **83,** 9254–9258.

Pert C. B., Smith C. C., Ruff M. R., and Hill J. M. (1988) AIDS and its dementia as a neuropeptide disorder: role of VIP receptor blockade by human immunodeficiency virus envelope. *Ann. Neurol.* **23,** S71–S73.

Philippe J., Drucker D. J., Knepel W., Jepeal L., Misulovin Z., and Habener J. F. (1988) Alpha-cell-specific expression of the glucagon promoter element by the interaction of DNA-binding proteins. *Mol. Cell. Biol.* **8,** 4877–4888.

Phillis J. W., Kirkpartick J. R., and Said, S. I. (1978) Vasoactive intestinal polypeptide excitation of central neurons. *Can. J. Physiol. Pharmacol.* **56,** 337–340.

Piper P. J., Said S. I., and Vane J. R. (1970) Effects on smooth muscle preparation of identified vasoactive peptides from intestine and lung. *Nature* **225,** 1144–1146.

Pless D. D. and Lennarz, W. J. (1977) Enzymatic conversion of proteins to glycoproteins. *Proc. Natl. Acad. Sci. USA* **74,** 134–138.

Poston G. J., Yao C. Z., Upp J. R., Alexander R. W., Townsend C. M., and Thompson J. C. (1988) Vasoactive intestinal peptide inhibits the growth of hamster pancreatic cancer but not human pancreatic cancer in vivo. *Pancreas* **3,** 439–443.

Pruss R. M., Moskal J. R., Eiden L. E., and Beinfeld M. C. (1985) Specific regulation of vasoactive intestinal polypeptide biosynthesis by phorbol ester in bovine chromaffin cells. *Endorcrinol.* **117,** 1020–1026.

Quik M., Iversen L. L., and Bloom S. R. (1978) Effect of vasoactive intestinal peptide (VIP) and other peptides on cAMP accumulation in rat brain. *Biochem. Pharmacol.* **27,** 2209–2213.

Rauscher F. J., III, Cohen D. R., Curran T., Bos T. J., Vogt P. K., Bohmann D., Tjian R., Franza B. R., Jr. (1988) Fos-associated protein p39 is the product of the june proto-oncogene. *Science* **240,** 1010–1016.

Reichlin S. (1988) Neuroendocrine significance of vasoactive intestinal polypeptide. *Ann. NY Acad. Sci.* **527,** 431–449.

Reid A. M., Shulkes A., and Titchen D. A. (1988) Effects of the vagus nerves on gastric motility and release of vasoactive intestinal polypeptide in the anaesthetized lamb. *J. Physiol.* **396,** 11–24.

Riabowol K. T., Fink J. S., Gilman M. Z., Walsh D. A., Goodman R. H., and Feramisco J. R. (1988) The catalytic subunit of cAMP-dependent protein kinase induces expression of genes containing cAMP responsive elements. *Nature* **336,** 83–86.

Robberecht P., De Neef P., Lammens M., Deschodt-Lanckman M., and Christophe J. P. (1978) Specific binding of vasoactive intestinal peptide to brain membranes of the guinea pig. *Eur. J. Biochem.* **90,** 147–154.

Robberecht P., Vandermeers A., Vandermeers-Piret M. C., Svoboda M., DeNeef P., DeGraef J., Woussen-Colle M. C., Yanaihara N., Yanaihara C., and Christophe J. (1985) Immunoreactive helodermin a new candidate neuropeptide in Mammalian. *Reg. Pep.* **3,** S17.

Robbins R. and Landon R. M. (1985) The effects of neurotensin, vasoactive intestinal polypeptide and other neuropeptides on the secretion of somatostatin from cerebral cortical cells. *Brain Res.* **332,** 161–164.

Rosselin G., Anteunis A., Astesano C., Boissard C., Boissard Gall P., Hejblum G., and Marie, J. C. (1988) Regulation of the vasoactive intestinal peptide receptor. *Ann. NY Acad. Sci.* **527,** 220–237.

Rostene W. H. (1984) Neurobiological and neuroendocrine functions of the vasoactive intestinal peptide (VIP). *Prog. Neurobiol.* **22,** 103–129.

Rotsztejn W. H., Benoist L., Besson J., Beraud G., Bluet-Pajot M. T., Kordon G., Rosselin G., and Duval J. (1980) Effect of vasoactive intestinal peptide (VIP) on the release of adenohypophyseal hormones from purified cells obtained by unit gravity sedimentation. *Neuroendocrinol.* **31,** 282–286.

Ruberg M., Rotszteijn W. H., Arancibia S., Besson J., and Enjalbert A. (1978) Stimulation of prolactin release by vasoactive intestinal peptide. *Eur. J. Pharmacol.* **51,** 319, 320.

Ruff M. R., Martin B. M., Ginns E. I., Farrar W. L., and Pert C.B. (1987) CD4 receptor binding peptides that block HIV infectivity cause human monocyte chemotaxis. Relationship to vasoactive intestinal peptide. *FEBS Lett.* **211,** 17–22.

Sacerdote P., Ruff M. R., and Pert C. B. (1987) Vasoactive intestinal peptide (1–12): A ligand for the CD4 (T4)/human immunodeficiency virus receptor. *J. Neurosci. Res.* **18,** 102–107.

Said S. I. and Mutt V. (1970) Polypeptide with broad biological activity: Isolation from small intestine. *Science* **169,** 1217, 1218.

Said S. I. and Faloona G. R. (1975) Elevated plasma and tissue levels of vasoactive intestinal polypeptide in the watery-diarrhea syndrome due to pancreatic bronchogenic and other tumors. *N. Engl. J. Med.* **293,** 155–160.

Said S. I. and Porter J. C. (1979) Vasoactive intestinal polypeptide: Release into hypophyseal portal blood. *Life Sci.* **24,** 227–230.

Said S. I., ed. (1982 Vasoactive Intestinal Polypeptide, *Advances in Peptide Hormone Research,* Raven, New York.

Said S. I. (1984) Isolation, localization and characterization of gastrointestinal peptides. *Peptides* **5,** 143–150.

Said S. I. (1986) Vasoactive intestinal peptide. *J. Endocrinol. Invest.* **9,** 191–200.

Said S. I. and Mutt V. (1988) Vasoactive intestinal peptide and related peptides. *Ann. NY Acad. Sci.,* **527.**

Samson W. K., Bianchi R., Mogg R. J., Rivier J., Vale W., and Melin P. (1989) Oxytocin mediates the hypothalamic action of vasoactive intestinal peptide to stimulate prolactin secretion. *Endocrinol.* **124,** 812–819.

Schmid R., Schusdziarra V., and Classen M. (1988) Modulatory effect of glucose on VIP-induced gastric somatostatin release. *Am. J. Physiol.* **254,** E756–759.

Schwartz J. P., Mocchetti I., Giorgi O., and Quach T. T. (1984) Transcriptional regulation of proenkephalin mRNA expression in striatum and adrenal medulla. *J. Cell. Biochem.* **8B,** 110.

Seylanz J., Hara H., Pinard E., Mraovitch S., and MacKenzie E. T. (1988) Effect of stimulation of the sphenopalatine ganglion on cortical blood flow in the rat. *J. Cereb. Blood Flow Metab.* **8,** 875–878.

Shaffer M. M. and Moody T. W. (1986) Autoradiographic visualization of CNS receptors for vasoactive intestinal peptide. *Peptides* **7,** 283–288.

Shimatsu A., Kato Y., Matsushita N., Katakami H., Yanaihara N., and Imura H. (1982) Stimulation by serotonin of vasoactive intestinal polypeptide release into rat hypophysial portal blood. *Endocrinol.* **111,** 338–340.

Sibony P. A., Walcott B., McKeon C., and Jakobiec F. A. (1988) Vasoactive intestinal polypeptide and the innervation of the human lacrimal gland. *Arch. Ophthalmol.* **106,** 1085–1088.

Silva C. M., Tully D. B., Petch L. A., Jewell C. M., and Cidlowski J. A. (1987) Application of a protein-blotting procedure to the study of human glucocorticoid receptor interactions with DNA. *Proc. Natl. Acad. Sci. USA* **84,** 1744–1748.

Silver R., Witkovsky P., Horvath P., Alones V., and Barnstable C. J. (1988) Coexpression of opsin- and VIP-like-immunoreactivity in CSF-contacting neurons of the avian brain. *Cell Tissue Res.* **253,** 189–198.

Simonosits A., Tjórnhammar M. L., Kalman M., Cserpan I., Gafvelin G., and Bartfai T. (1988) Synthesis, cloning and expression in Escherichia coli of artificial genes coding for biologically active elongated precursors of the vasoactive intestinal polypeptide. *Eur. J. Biochem.* **178,** 343–350.

Singh H., Kumar A., Townsend C. M., Samad Z., and Singh P. (1988) A synthetic peptide, L-8-K, and its antibody both inhibit the specific binding of vasoactive intestinal peptide to hamster pancreatic cancer cells. *Ann. NY Acad. Sci.* **527,** 679–681.

Southern E. M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98,** 503–517.

Speigel A. M. (1987) Signal transduction by guanine binding proteins. *Mol. Cell. Endocrinol.* **49,** 1–16.

Spokes R. A., Yiangou Y., Chrysanthou B. J., Bowles M. P., and Bloom S. R. (1989) Effects of prepro-vasoactive intestinal polypeptide-derived peptides on net fluid flux in small intestine of anesthetized rats. *Gastroenterol.* **96,** 327–330.

Stanisz A. M., Befus D., and Bienenstock J. (1986) Differential effects of vasoactive intestinal peptide, substance P and somatostatin on immunoglobulin synthesis and proliferation by lymphocytes from Peyer's patches, mesenteric lymph nodes and spleen. *J. Immunol.* **136,** 152–156.

Staun-Olsen P., Fahrenkrug J., Gammeltoft S., Ottesen B., and, Schousboe A. (1985) Development of binding sites for vasoactive intestinal polypeptide in mouse cerebral cortex and cultured cortical neurons. *Int. J. Dev. Neurosci.* **3,** 609–616.

Steiner D. F., Quinn P. S., Chan S. J., Marsh J., and Tager H. S. (1980) Proceedings mechanisms in the biosynthesis of proteins. *Ann. NY Acad. Sci.* **343,** 1–16.

Stevens L. M. and Landis S. C. (1988) Developmental interactions between sweat glands and the

sympathetic neurons which innervate them: Effects of delayed innervation on neurotransmitter plasticity and gland maturation. *Dev. Biol.* **130,** 703–720.

Stone R. A., Laties A. M., Raviola E., and Wiesel T. N. (1988) Increase in retinal vasoactive polypeptide after eyelid fusion in primates. *Proc. Natl. Acad. Sci. USA* **85,** 257–260.

Suzuki N., Hardebo J. E., and Owman C. (1988) Origins and pathways of cerebrovascular vasoactive intestinal polypeptide-positive nerves in rat. *J. Cereb. Blood Flow Metab.* **8,** 697–712.

Svoboda M., De Neef P., Tastenoy M., and Christophe J. (1988) Molecular characteristics and evidence for internalization of vasoactive intestinal peptide (VIP) receptors in the tumoral rat-pancreatic acinar cell line AR 4-2 J. *Eur. J. Biochem.* **176,** 707–713.

Tapia-Arancibia L. and Reichlin S. (1985) Vasoactive intestinal peptide and PHI stimulate somatostatin release from rat cerebral cortical - diencephalic cells in dispersed culture. *Brain Res.* **336,** 67–72.

Tapia-Arancibia L., Pares-Herbute N., Astier H., Reichlin S., and Nathanson J. (1988) Adenylate cyclase activation is not sufficient to stimulate somatostatin release from dispersed cerebral cortical and diencephalic cells in glia-free cultures. *Brain Res.* **450,** 101–110.

Tatemoto K. and Mutt V. (1980) Isolation of two novel candidate hormones using a chemical method for finding naturally occurring peptides. *Nature* **285,** 417, 418.

Taylor D. and Pert C. B. (1979) Vasoactive intestinal polypeptide: Specific binding to rat brain membranes. *Proc. Natl. Acad. Sci. USA* **76,** 660–664.

Thomas G. B., Cummins J. T., Griffin N., and Clarke I. J. (1988) Effect and site of action of hypothalamic neuropeptides on prolactin release in sheep. *Neuroendocrinol.* **48,** 252–257.

Thompson D. C., Altiere R. J., and Diamond L. (1988) The effects of antagonists of vasoactive intestinal peptide on nonadrenergic noncholinergic inhibitory responses in feline airways. *Peptides* **9,** 443–447.

Tornell J., Carlsson, B., and Hillensjo, T. (1988) Vasoactive intestinal peptide stimulates oocyte maturation, steroidogenesis, and cyclic adenosine 3',5'-monophosphate production in isolated preovulatory rat follicles. *Biol. Reprod.* **39,** 213–220.

Towbin H., Staehelin T., and Gordun J. (1979) Electrophoretic transfer of protein's from polyacrylamide gels to nitrocellulose sheets: Procedure and some application. *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.

Tricoli J. V., Bell G. I., and Shows T. B. (1984) The human glucagon gene is located on chromosome 2. *Diabetes* **33,** 200–202.

Tsukada T., Fink J. S., Mandel G., and Goodman R. H. (1987) Identification of a region in the human vasoactive intestinal polypeptide gene responsible for regulation by cyclic AMP. *J. Biol. Chem.* **262,** 8743–8747.

Tsukada T., Horovitch S. J., Moutminy M. R., Mandel G., and Goodman R. H. (1985) Structure of the human vasoactive intestinal polypeptide gene. *DNA* **4,** 293–300.

Turner J. T., Jones S. B., and Bylund D. B. (1986) A fragment of vasoactive intestinal peptide, VIP (10–28), is an antagonist of VIP in the colon carcinoma cell line, HT29. *Peptides* **7,** 849–854.

Turner J. T., Bollinger D. W., and Toews M. L. (1988) Vasoactive intestinal peptide receptor/adenylate cyclase system: Differences between agonist- and protein kinase C-mediated desensitization and further evidence for receptor internalization. *J. Pharmacol. Exp. Ther.* **247,** 417–423.

Uemura Y., Sugimoto T., Kikuchi H., and Mizuno N. (1988) Possible origins of cerebrovascular nerve fibers showing vasoactive intestinal polypeptide-like immunoreactivity: An immunohistochemical study in the dog. *Brain Res.* **448,** 98–105.

Van den Pol A. N. and Powley T. (1979) A fine-grained anatomical analysis of the role of the rat suprachiasmatic nucleus in circadian rhythms of feeding and drinking. *Brain Res.* **160,** 307–326.

Van den Pol A. N. and Tsujimoto K. L. (1985) Neurotransmitters of the hypothalamic suprachiasmatic nucleus: Immunocytochemical analysis of 25 neuronal antigens. *Neuroscience* **15,** 1049–1086.

Van den Pol A. N., and Gorcs T. (1986) Synaptic relationships between neurons containing vasopressin, gastrin-releasing peptide, vasoactive intestinal polypeptide, and glutamate decarboxylase immunoreactivity in the suprachiasmatic nucleus: Dual ultrastructural immunocyto-

chemistry with gold-substituted silver peroxidase. *J. Comp. Neurol.* **252,** 507–521.

Venugopalan C. S., Holmes E., and Pillai S. R. (1989) Blockade of NANC inhibitory tracheal response by peptide T. *FASEB J.* **3,** A287.

Vincent S. R. and Reiner P. B. (1988) A population of very small striatal neurons in the cat displays vasoactive intestinal polypeptide immunoreactivity. *Neurosci. Lett.* **89,** 277–282.

Waelbroeck M., Robberecht P., Coy D. H., Camus J. C., Deneef P., and Christophe J. (1985) Interaction of growth hormone-releasing factor (GRF) and 14 GRF analogs with vasoactive intestinal peptide (VIP) receptors of rat pancreas. Discovery of (N-Ac-Tyr^1,D-Phe^2)-GRF(1–29)—NH_2 as a VIP antagonist. *Endocrinol.* **116,** 2643–2649.

Wang Y.-Y. and Aghajanian G. K. (1988) VIP excitation of locus coeruleus neurons: Evidence for a G-protein-mediated inward current. *Soc. Neurosci. Abs.* **14,** 147 (Abs. 59.9).

Waschek J. A. and Eiden L. E. (1988) Calcium requirements for barium stimulation of enkephalin and vasoactive intestinal biosynthesis in adrenomedullary chromaffin cells. *Neuropeptides* **11,** 39–45.

Waschek J. A., Hsu C.-M., and Eiden L. E. (1988) Lineage-specific regulation of the vasoactive intestinal peptide gene in neuroblastoma cells is conferred by 5.2 kilobases of 5′-flanking sequence. *Proc. Natl. Acad. Sci. USA* **85,** 9547–9551.

Wattchow D. A., Furness J. B., and Costa M. (1988) Distribution and coexistence of peptides in nerve fibers of the external muscle of the human gastrointestinal tract. *Gastroenterol.* **95,** 32–41.

Watts A. G. and Swanson L. W. (1987) Efferent projections of the suprachiasmatic nucleus: II. Studies using retrograde transport of fluorescent dyes and simultaneous peptide immunocytochemistry in the rat. *J. Comp. Neurol.* **258,** 230–252.

Weinstein J., Pope M., Schmidt R., and Seroussi R. (1988) Neuropharmacologic effects of vibration on the dorsal root ganglion. An animal model. *Spine* **13,** 521–525.

Werner H., Koch Y., Fridkin M., Fahrenkrug J., and Gozes I. (1985) Detection of elevated amounts of VIP in milk. *Biochem. Biophys. Res. Commun.* **136,** 228–232.

Werner H., Koch Y., Baldino F., Jr., and Gozes I. (1988) Estrogen regulation of somatostatin mRNA in the rat hypothalamus. *J. Biol. Chem.* **263,** 7666–7671.

White J. D. and Gall C. M. (1987) Differential regulation of neuropeptide and proto-oncogene mRNA content in the hippocampus following recurrent seizures. *Mol. Brain Res.* **3,** 21–29.

Wiik P. (1988) Homologous regulation of adenylate cyclase-coupled receptors for vasoactive intestinal peptide (VIP) on human mononuclear leukocytes. *Reg. Pept.* **20,** 323–333.

Wynick D., Williams S. J., and Bloom S. R. (1988) Symptomatic secondary hormone syndromes in patients with established malignant pancreatic endocrine tumors. *N. Engl. J. Med.* **319,** 605–607.

Yada T. and Okada Y. (1984) Electrical activity of an intestinal epithelial cell line; hyperpolarizing responses to intestinal secretagogues. *J. Membrane Biol.* **77,** 33–44.

Yaksh T. L., Michener S. R., Bailey J. E., Harty G. J., and Lucas D. L. (1988) Survey of distribution of substance P, vasoactive intestinal polypeptide, cholecystokinin, neurotensin, Met-enkephalin, bombesin and PHI in the spinal cord of cat, dog, sloth and monkey. *Peptides* **9,** 357–372.

Yamagami T., Ohsawa K., Nishizawa M., Inoue C., Gotoh E., Yanaihara N., Yamamoto H., and Okamoto H. (1988) Complete nucleotide sequence of human vasoactive intestinal peptide/PHM-27 gene and its inducible promoter. *Ann NY Acad. Sci.* **527,** 87–102.

Yu G., Grant S., and Glazer R. I. (1988) Association of p93c-fes tyrosine protein kinase with granulocytic/monocytic differentiation and resistance to differentiating agents in HL-60 leukemia cells. *Mol. Pharmacol.* **33,** 384–388.

Yuwiler A. and Wetterberg L. (1989) Peptide T does not affect induction of pineal *N*-acetyltransferase by vasoactive intestinal peptide. *Regul. Pept.* **25,** 69–73.

Zurier R. B., Kozma M., Sinnett-Smith J., and Rozengurt E. (1988) Vasoactive intestinal peptide synergistically stimulate DNA synthesis in mouse 3T3 cells: Role of cAMP, Ca2+ and protein kinase C. *Exp. Cell Res.* **176,** 155–161.