Vasoactive Intestinal Peptide in Neurodevelopmental Disorders: Therapeutic Potential

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Abstract: Vasoactive intestinal peptide (VIP) mediates important events during the development of the nervous system. VIP can stimulate neuronogenesis as well as differentiation and neurite outgrowth; it can promote the survival of neurons and assist in neuronal repair; it is also anti-inflammatory and can modulate immune responses. In addition, VIP is necessary for the normal growth and development of the early postimplantation mouse embryo during the period when the major embryonic events are neural tube formation, neuronogenesis and expansion of the vascular system. Receptors for VIP appear during early postimplantation embryogenesis in the rodent and exhibit changing localization patterns throughout the development of the brain. During embryogenesis, unregulated VIP may have major and permanent consequences on the formation of the brain and may be a participating factor in disorders of neurodevelopment. VIP has been linked to autism, Down syndrome and fetal alcohol syndrome. This paper will review the role of VIP in neurodevelopment, its known involvement in neurodevelopmental disorders and propose ways in which VIP might be of therapeutic value.

Key Words: Embryogenesis, Autism, Down syndrome, Fetal alcohol syndrome.

INTRODUCTION

Vasoactive intestinal peptide (VIP) is a 28 amino acid neuropeptide originally isolated from the gut [1] and subsequently found to be widely distributed within the peripheral and central nervous systems [2-5]. VIP receptors occur early in rodent embryogenesis [6-9] and exhibit dynamic patterns of localization in the nervous system throughout both pre- and postnatal development [6]. VIP mRNA begins to appear a number of days after the appearance of VIP receptors in the mouse [9,10] and the adult pattern of distribution of VIP mRNA in rodent brain is gradually expressed throughout development [6, 11]. The origin of VIP and its receptors during embryogenesis indicates that it may be involved in several aspects of neurobehavioral development. VIP has recognized roles in the nervous, immune, respiratory, circulatory, endocrine and digestive systems where it has neurotransmitter and neuromodulator functions. VIP has broad actions; it is known to promote neurite outgrowth and is neuroprotective, proliferative, neurotrophic [10, 12-21], immunomodulatory [24, 25] and, in rodents, VIP has been shown to regulate growth and development during embryogenesis [6-9, 26, 27]. Peripherally, VIP influences many functions including blood flow and cardiac output, smooth muscle activity, secretion in the digestive tract, gastric motility, bronchodilation [4] and activity within the hypothalamic-pituitary-adrenal axis [28]. Recent work has revealed that VIP has important functions in immunomodulation with possible cytokine-like action, and, although VIP is localized to neurons throughout the body, it has also been identified in lymphoid cells including leukocytes, eosinophils, mast cells and lymphocytes [24, 25]. In the brain, VIP and its receptors are widely distributed and the neurotransmitter and neuromodulatory activities of VIP are broad and include such diverse actions as rhythm generation in the suprachiasmatic nucleus [29, 30], the regulation of neuroendocrine secretions in the hypothalamus [31] and energy metabolism of glial cells [32]. VIP is released from neurons upon depolarization, and is known to stimulate adenylyl cyclase. It regulates several functions indirectly through the stimulation of the release of factors from other cells, particularly the release of glial factors in the brain [33, 38]. VIP belongs to a family of peptides that includes pituitary adenylyl cyclase activating peptide (PACAP), the family member with which it shares the greatest homology and with which it is sometimes co-localized [39]. There is some overlap of function between VIP and PACAP, probably through action at the two known G protein coupled receptors, VPAC1 and VPAC2, which they share and to which they have similar affinity. PACAP binds with high affinity to a third receptor, PAC1, for which VIP has only a low affinity [40-42]. VPAC 1 and VPAC2 have different distribution patterns in the brain [43, 44]. Evidence for the existence of additional VIP receptors, or VIP receptor subtypes, is increasing and further exploration of these sites may clarify the differential effects of VIP, PACAP and related peptides in physiological functions [45]. VIP and a second peptide, peptide histidine isoleucine (PHI), originate from the same gene [46], the same precursor peptide, exhibit similarities in distribution, and are co-released upon activation. VIP and PHI are believed to have distinct functions and recently receptors with a high affinity for PHI have been discovered [45, 47, 48].

VIP IN DEVELOPING CENTRAL NERVOUS SYSTEM TISSUES

Mitogenesis, Differentiation and Neurite Outgrowth

VIP is known to regulate a number of developmentally important events at the cellular level including mitogenesis,

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differentiation, neuron outgrowth, neuronal survival and protection. Dysregulation of VIP during these processes could form a cellular/molecular basis of disorders in neurodevelopment.

VIP exhibits differential effects on mitogenesis and differentiation in different populations of cells. In several culture systems, including neuroblastoma cells, neuroendocrine cells, lymphocytes and splenocytes, VIP has been shown to inhibit mitogenesis and induce differentiation [18, 19]. However, VIP has proliferative properties and can stimulate mitogenesis in neuroblasts [15, 17, 21], astrocytes and neurons [14]. In embryonic stem cells, VIP can induce the differentiation of neurons without affecting the proportion of glial cells [49]. VIP stimulates neurite outgrowth and sprouting of spinal cord neurons [20, 22] and sympathetic neuroblasts [15]. Whereas VIP promotion of neuronal differentiation and neurite outgrowth occurs in the micromolar/nanomolar range, the related peptide PACAP has more potent effects [50], suggesting that, during development, some adenylyl cyclase-dependent events may be preferentially mediated through the PACAP preferring PAC1 receptor.

Neuronal Survival and Protection

The neurotrophic actions of VIP were first demonstrated in embryonic spinal cord cells [12] where, at subnanomolar concentrations, VIP was shown to increase the survival of electrically blocked neurons. Subsequent studies with spinal cord, cerebral cortex and hippocampal cells showed that the presence of glial cells was necessary for VIP to promote the survival of neurons exposed to neurotoxic substances, or if the actions of endogenous VIP were blocked [13, 16]. Further, these studies showed survival-promoting actions of VIP at picomolar concentrations mediated by the mobilization of calcium and phosphokinase C isozymes [51]. In contrast, in the peripheral nervous system, the survival-promoting actions of VIP require higher concentrations, are mediated by cAMP, and PACAP may be the primary ligand regulating these functions [50].

A role for VIP in repair following neuronal injury is supported by several studies. Following sensory or sympathetic nerve transection or crushing, an increase of both VIP [52-54] and VIP mRNA [52, 55] were seen.

INDIRECT ACTIONS OF VIP

The stimulation of high affinity VIP receptors on astrocytes [16, 56] has been shown to result in the release of a number of proteins including cytokines [33], protease nexin 1 [34], chemokines [35], activity dependent neurotrophic factor (ADNF) [36] and activity dependent neuroprotective protein (ADNP) [37, 38]. Thus, many of the protective and repair mechanisms of VIP may be due to its stimulation of the release of other factors. For example, VIP stimulates the release of the potently neuroprotective protein, ADNF, which prevents neuronal cell death associated with such toxins as gp120 (the envelope protein of HIV), N-methyl d-aspartate, beta amyloid peptide and tetrodotoxin [36]. NAP, the active peptide of ADNP, also exhibits neuroprotective properties against insults such as glucose deprivation and N-methyl d-aspartate, gp120 and tumor necrosis factor alpha toxicity [57]. The differentiation-promoting action of VIP may be due to the VIP induced release of cytokines [58]. Also, VIP increases the expression [59] and release of protease nexin 1 that, in turn, stimulates neurite outgrowth following nerve lesion.

The anti-inflammatory properties of VIP might also be considered indirect actions as VIP inhibits the production of the proinflammatory cytokines tumor necrosis factor alpha, interleukin (IL)-6, IL-12 and of nitric oxide, and stimulates the production of the anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist. These actions are believed to occur through the differential use of VIP receptors acting through different intracellular signal pathways [see review 25].

VIP IN EMBRYONIC GROWTH AND NEURODEVELOPMENT

The first indication that VIP had a role in embryogenesis was in experiments by Gressens *et al*. [26] in which whole cultured embryonic day (E) 9.5 mouse embryos exhibited dramatic, dose-dependent growth following treatment with VIP in a dose range of 10^{-11}M to 10^{-7}M. The embryos grew in a coordinated manner with increases in DNA, protein, and cross sectional area and an increase in somite number double that seen in untreated embryos. Further experiments showed that treatment with PACAP had no effect on growth at low concentrations but inhibited growth at higher concentrations, indicating a specificity of VIP actions on growth stimulation [8] (Fig. **1**). Treatment of pregnant mice with an antagonist to VIP during this period of development (E9-E11) resulted in microcephaly and growth restriction. Blockage of VIP after E11 did not restrict growth or development, indicating that the VIP effects occurred during a narrow window of time [27]. Taken together, these experiments showed that VIP was necessary for normal growth and development of the mouse embryo during early postimplantation embryogenesis at neural tube closure.

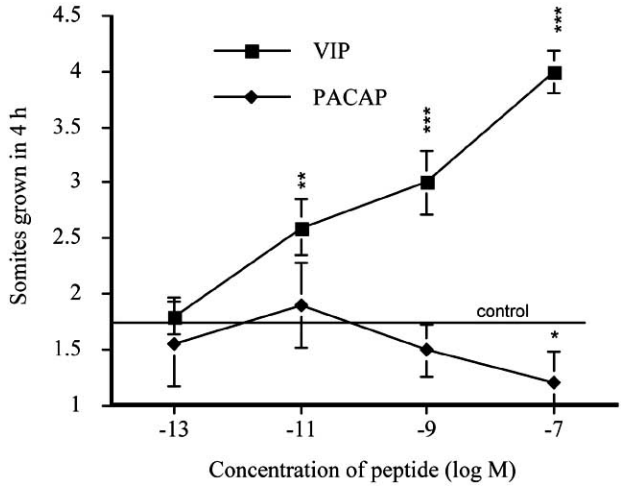

Fig. (1). Mean somites grown ±S.E.M. in 4-h incubation of E9.5 mouse embryos. Treated embryos were cultured with 10^{-13}, 10^{-11}, 10^{-9} and 10^{-7} M VIP and PACAP. A minimum of two experiments was performed for each treatment group. VIP-treated embryos grew significantly more somites than controls at 10^{-11}M, 10^{-9}M and 10^{-7}M. PACAP-treated embryos grew significantly fewer somites than controls at 10^{-7} M. Reproduced, in part, with permission [8].

In the mouse embryo, VIP binding sites were first noted at E9 and were primarily localized to the floor plate of the newly formed neural tube [8, 9] (Fig. **2**). The floor plate is composed of glial cells and is a recognized organizing center of the developing CNS through the release of soluble factors, such as sonic hedgehog protein [60].

Although VIP peptide can be measured in the E9 mouse embryo, VIP mRNA was not detectable in the embryo until E11 [9]. However, beginning as early as E6, the uterine decidua of the pregnant female (the maternal tissue with which the embryonic trophoblasts and vasculature come into intimate contact forming a locus for maternal/embryonic exchange) was enriched with immunoreactive VIP, VIP mRNA and VIP binding sites [9]. VIP was localized to gamma, delta T lymphocytes in the mouse decidua [9]. This maternal immune cell forms an enriched and expanded population at the maternal/embryo-fetal interface, can recognize and react to trophoblasts, and, among other things, mediates placental development and immunosuppression [61]. Although decidual VIP and VIP binding sites may have additional roles in early pregnancy, at least by E9 it appears that maternal VIP reaches the embryo where it could act on neural tube binding sites to regulate a coordinated growth of the mouse embryo brain and body.

The embryonic period of E8 – 11 in the mouse is characterized by the transition from yolk sac to chorioallantoic placental nutrition and, along with implantation (E4.5 - 6), comprises the most vulnerable period of prenatal development. More than 20% of human pregnancies are lost in early pregnancy [62] and similar losses occur in domesticated animals [63]. During E9–11 in the mouse, while growth is under the regulation of VIP, the major nervous system events include neural tube closure, neuronogenesis, optic, olfactory and otic placode formation and formation of the cranial nerves. By E10, organogenesis of other organ systems is well underway [64].

Although VIP-induced growth occurs through a shortening of G1 and S phases of the cell cycle [65], studies have indicated that VIP regulates growth, at least in part, by stimulating the actions of ADNF and insulin-like growth factor 1 (IGF-1) [66, 67]. ADNF dramatically stimulates growth in whole cultured E9.5 mouse embryos at concentrations as low as 10^{-13}M, and antibodies to ADNF inhibit VIP-induced growth [67]. Furthermore, VIP appears to directly, or indirectly through ADNF, regulate the action of IGF-1 [67] and perhaps other growth factors, which speed the rate of cell division and growth. IGF-1 is a well-recognized growth-regulating factor from embryogenesis to postnatal development [68-70]. Nerve growth factor (NGF) is also among factors regulated by VIP in the mouse embryo. Concentrations of VIP within its biological range stimulate the release of a 60-kDa prohormone form of NGF from the neural tube in concentrations within the range of the biological actions of NGF [71].

Blocking the action of VIP with a VIP antagonist for three days during embryogenesis apparently causes permanent changes in the CNS [72, 73]. To begin to understand the role of VIP in nervous system development we examined the effects of blocking VIP action with the VIP hybrid antagonist (VA) [16] during the early postimplantation developmental period, including E9–11 in the mouse [71-73] and also during postnatal development in the rat [74]. Neonatal mice that had experienced a blockage of VIP as embryos revealed a dose-dependent retardation in the appearance of 5 of 10 developmental milestones [72], including eye opening and several reflexes. Mice experiencing blockage of PACAP with PACAP 6-28, did not exhibit retardation of developmental signs (Table **1**). VA-treated pups were significantly smaller at birth and growth restriction was still apparent at weaning (Fig. **3**). VIP antagonist treatment of neonatal rats did not significantly retard the growth of the brain or body. However, developmental milestones were delayed [74], VIP and VIP binding sites were increased in the brain, and neu-

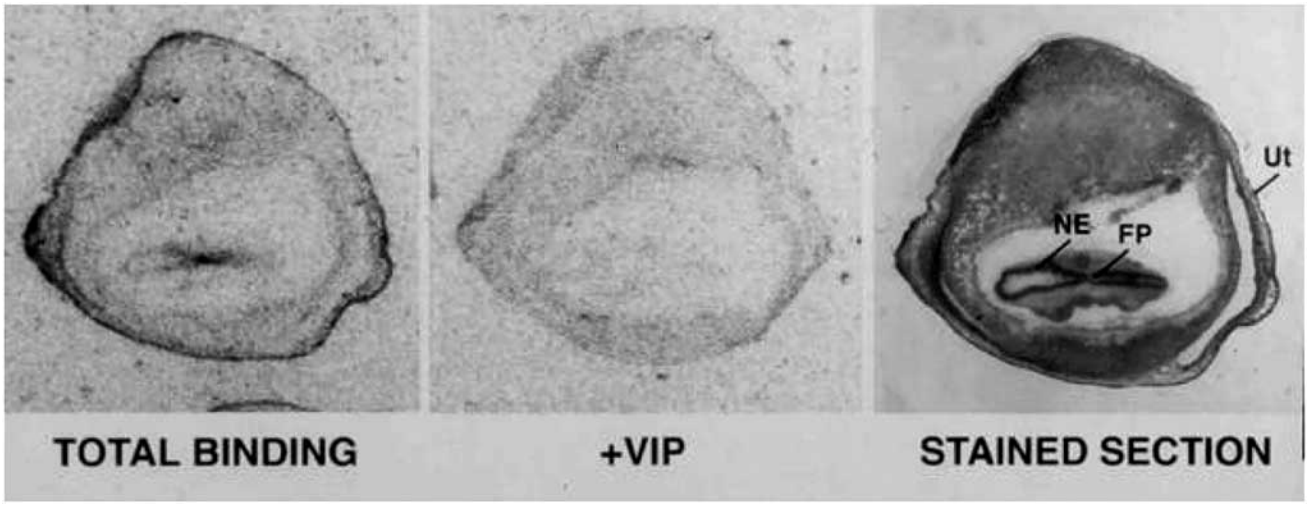

Fig. (2). Film autoradiography of [^{125}I] VIP binding in the E9 pregnant mouse uterus. +VIP = [^{125}I] VIP binding in the presence of 10^{-6}M VIP. Stained section = the section appearing in 'Total binding' following staining in hematoxylin. FP = floor plate; NE = neuroepithelium; Ut = uterus. Reproduced, in part, with permission [8].

Table 1. Neonatal Developmental Milestones. Neonatal Mice were Tested Daily for the First 21 Days of Life for a Number of Reflexes and Developmental Signs as Previously Described [68]

Behavior	Control	VIP antagonist#	PACAP antagonist##	F(p)
Weight in grams P2	1.9±0.03	1.7±0.03***	1.9±0.23	21 (0.0001)
Surface Right first day <1 sec	5.3±0.33	8.4±0.26***	5.4±0.24	23.8 (0.0001)
Negative Geotaxis first day	3.8±0.19	3.8±0.35	3.0±0.21	3.47 (0.051)
Cliff Aversion first day	4.3±0.25	4.2±0.34	3.8±0.13	1.13 (0.34)
Rooting first day	4.6±0.2	4.6±0.1	4.5±0.3	0.11 (0.90)
Grasping first day > 1 sec	8.3±0.3	10.3±0.1***	7.4±0.4	15.8 (0.0001)
Open field first day	14.7±0.4	18.5±0.3***	14.2±0.3	34.1 (0.0001)
Auditory Startle first day	10.8±0.3	11.7±0.1*	10.5±0.3	3.7 (0.042)
Ear Twitch first day	10.0±0.1	10.5±0.2	9.7±0.2	2.7 (0.09)
Eye Opening first day	13.4±0.1	13.6±0.2	13.7±0.1	2.8 (0.08)
Air righting first day	10.9±0.1	11.3±0.3	11.1±0.2	1.08 (0.35)

#VIP antagonist = VIP hybrid antagonist [14, 68] injected twice daily E8-E10, 30 μg/day.
##PACAP antagonist = PACAP (6-38), injected twice daily E8-E10, 30 μg/day.
*= p<0.05; *** = p<0.0001.

rons in the adult cortex exhibited neuronal dystrophy [75]. Cortical neurons had fewer, shorter and thicker dendrites that took tortuous paths. Dendritic spines were reduced in number, enlarged, fused and vacuolated.

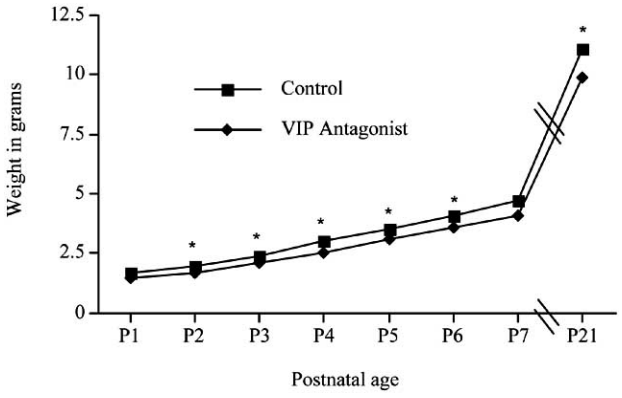

Fig. (3). Weight in grams of postnatal mouse pups. VIP antagonist-treated pups experienced blockage of VIP with a VIP hybrid antagonist (30 μg/day)[14], injected twice daily, E8-E10, as previously described [68]. P = postnatal day. VIP antagonist treated pups were significantly growth restricted from P2 to P21, *p<0.05.

The genesis of the nervous system is extremely complex and there are many remaining questions regarding how the intricate, coordinated events involved in its formation are synchronized. However, much evidence has accrued indicating broad roles for VIP in neurodevelopment, beginning in early embryogenesis, and that dysregulation of this neuropeptide may result in permanent changes in neuroanatomy, neurochemistry and behavior.

VIP IN NEURODEVELOPMENTAL DISORDERS

VIP in Autism

Autism is a neurodevelopmental disorder recognized by three defining characteristics: 1) impairment of social behavior; 2) impairment of language, communication and imaginative play; and 3) repetitive behaviors and narrowly focused interests. A number of secondary symptoms may be expressed in persons with autism including: hypersensitivity, hearing dysfunction [76, 77], mental retardation, cognitive dysfunction, anxiety, seizures, neuropathologies, gastric immunopathology [78-80], sleep disorders [81-83] and an increased inflammatory response [84].

Although autism has been linked to environmental factors, it also has a strong genetic component. Environmental factors are suggested because of the reports of autism following thalidomide [85] or valproic acid exposures [86-88] providing injury at the time of closure of the neural tube. Other suggested factors include prenatal alcohol exposure [89], infections and vaccination [90, 91]. Mercury in childhood vaccinations has been put forth as a causative factor in autism [92]; however, this remains a controversial concept [93], in part because the potential toxin is administered postnatally. The very high concordance rates for monozygotic twins (80%) and the gender ratio of male to female of 4 to 1 strongly indicate a genetic component. Even among discordant twins, the co-twin often exhibits mild impairment [94]. Genomic screens have identified several genes that may be associated with autism [95], but it appears that susceptibility to autism may be due to multiple interacting genes with phenotypic expression triggered and modified by environmental factors [96].

Neuroanatomical studies of autistic patients have revealed a larger than normal brain volume in early childhood

of autistic patients, followed by low volumes in adolescence [97]. In addition, impaired connectivity [98], lower white matter volume in frontal, temporal and occipital lobes [94], cerebellar hypoplasia and decreases in Purkinje cells are reported [99, 100]. Lesions of motor cranial nerve nuclei, including the absence of facial nucleus and superior olive, and the shortening of brainstem (which would only arise if insult occurred during neural tube closure) have also been found [101,102]. Consistent with the hypothesis that the insult occurs during embryogenesis is the increased frequency of variants of *HOXA1* and *HOAXBa* alleles, two genes critical to hindbrain development, in a population of autistic patients [103]. Neurochemical differences include reports of hyperserotoninemia [104, 105] and variant alleles for the serotonin transport gene are reported in autistic populations [106]. Levels of the neuropeptides VIP, calcitonin gene-regulating peptide, brain-derived neurotrophic factor and neurotrophin 4/5, were higher than normal in the blood of newborns later found to be autistic or mentally retarded [107]. Secretin, a peptide belonging to the same family as VIP and PACAP, has been implicated in autism. Although not supported by further studies [108], Horvath and colleagues initially observed that autistic children treated with secretin exhibited improvement in mental functions [109]. Secretin is well recognized as a gut peptide [110]; however, recent immunohistochemical studies localizing secretin to the hypothalamus and other regions of the brain [111, 112] suggest that the full range of actions of this peptide is not fully understood.

In the search for animal models of autism, prenatal insults or genetic modifications that influence the development of the nervous system have been examined. Animal models of the neuroanatomical deficits seen in autism include brainstem and cerebellar anomalies following valproic acid treatment [113]. In addition, behavioral alterations of these animals included lowered pain sensitivity [114], diminished prepulse inhibition, reduced social interaction and increased stereotypies [115], all of which are also found in autism. Oxytocin and vasopressin have recognized roles in social attachment behavior [116] and vasopressin VI A receptor knockout mice have a profound impairment of social recognition and a reduction in anxiety-like responses [117]. Neonatal Borna disease virus-induced brain injury of neonatal rats produces animals that exhibit several autistic-like neuropathological and behavioral deficits [118].

Several factors suggest a link between VIP and autism. 1) As mentioned above, higher concentrations of VIP were found in the blood samples of newborn babies that were subsequently found to have autism [107]. 2) In the rodent, VIP is an important regulator of development during the period of neural tube closure [6-9, 26, 27] and there is compelling evidence that this is the period in human development in which the disordered neural development resulting in autism is initiated [86-88, 101-103]. 3) A subset of persons with autism are afflicted with a distinct form of inflammatory bowel disorder [78, 79] and VIP is not only an important gut peptide, regulating motility and secretion, but also an important immunomodulatory factor [24, 25]. 4) There is an increased production of proinflammatory cytokines reported in autism [84], and VIP is known to inhibit proinflammatory cytokine production and stimulate anti-inflammatory cytokines production [24, 25]. 5) Disordered sleep-wake cycles have been reported in persons with autism [81-83], and VIP is an important peptide in the suprachiasmatic nucleus of the hypothalamus where it is essential for the maintenance of sustained rhythm generation [119]. In addition, VIP in the amygdala has been implicated in rapid eye movement sleep [120] and rapid eye movement sleep disorder has been reported in children with autism [121]. 6) Results from examination of polymorphisms in the upstream region of the VPAC2 receptor gene suggest a potential link between this gene and the gastrointestinal and stereotypical behaviors in autistic persons [122].

Collectively, these factors, plus the microcephaly, growth restriction and developmental delays seen in neonatal mice in which VIP has been blocked during embryogenesis [27, 73, 74], indicate that this paradigm may be a useful model in furthering the understanding of developmental disorders in humans. In recent experiments we have found that mice experiencing a blockage of VIP during neural tube closure, the period during which VIP regulates growth and development, exhibited deficits in social behavior [123]. Further examination of this model is necessary to determine if it can become a model for disorders of social behavior, including autism.

VIP in Trisomy

Down syndrome is due to an additional copy of chromosome 21 in humans and is the most common known genetic cause of mental retardation [124]. It is characterized by growth restriction, developmental delays, cognitive dysfunction [125], as well as facial dysmorpology, immunodeficiencies, male infertility, and cardiac and intestinal defects [126]. Neuropathologies include dystrophic neurons and dendritic spines, smaller numbers and sizes of neurons [126, 127], astrocyte hypertrophy and increased numbers of astrocytes [128]. Down syndrome patients eventually exhibit the neuropathology of Alzheimer disease [129, 130].

The segmental trisomy mouse (Ts65Dn) exhibits several characteristic features of Down syndrome, including growth and developmental delays [131, 132], cognitive dysfunction [133-135], facial dysmorphy [136], neuronal and glial malformations [131, 137] male infertility [138] and degeneration of the cholinergic forebrain system [132, 139, 140].

Mice that have experienced blockage of VIP during embryogenesis or neonatal development exhibit several characteristics similar to both Down syndrome and the segmental trisomy mouse model of Down syndrome. For example, neonatal mice in which VIP was blocked during embryogenesis [72], or neonatal rats during the first two postnatal weeks [74], exhibited hypotonia, growth restriction and developmental delays. Also, spine dystrophy was seen in the cortical neurons of the brains of adult rats that had experienced a blockage of VIP during postnatal development [75]. Learning and memory deficits were seen in adult rats treated introcerebroventricularly with a VIP antagonist [141]. In addition, astrocytes from the cortex of segmental trisomy mice exhibited abnormal responses to treatment with VIP [142]. Our recent studies have shown that VIP biochemistry is dysregulated in the brains of segmental trisomy mice and, compared with their wild type littermates, segmental trisomy mice had significantly more VIP binding sites, VIP mRNA and VIP-immunopositive cells [143].

The results discussed above indicate that dysfunction of VIP may be a factor in neurodevelopmental disorders accompanied by mental retardation. Although a genetic/molecular/physiological link between the triplication in Ts65Dn and VIP is not apparent, the VIP abnormalities appear to be a response to subsequent pathological changes induced by the triplicated genes. Similar pathological changes in VIP may be a feature of other neurodevelopmental disorders since VIP levels are increased not only in Down syndrome newborns but also in those subsequently exhibiting autism [107].

VIP in Fetal Alcohol Syndrome

The frequency of fetal alcohol syndrome in the United States is 0.5 to 3 per 1000 births per year [144], and maternal alcohol consumption is the most commonly identified non-genetic cause of mental retardation [145]. As well as characteristic facial features and cranio-facial dysmorphology, children with fetal alcohol syndrome have microcephaly, growth restriction and central nervous system damage. Our finding that blockage of VIP during mouse embryogenesis also results in microcephaly, growth restriction and developmental delays [26, 72], suggests that an alcohol-induced imbalance of VIP during embryogenesis may account for some aspects of fetal alcohol syndrome. This concept is supported by additional studies in which alcohol exposure during embryonic day 8 in the mouse caused a reduction in VIP and VIP mRNA in the uterus (decidua, membranes and embryo) [146]. In this study, cotreatment with the active peptides of proteins ADNF and ADNP, which are regulated by VIP, prevented alcohol-induced fetal death and growth abnormalities [146]. There are other known links between VIP and alcohol. VIP mRNA is decreased in the suprachiasmatic nucleus with chronic alcohol use [147], and alcohol inhibits alpha adrenergic potentiation of VIP-regulated cAMP and cGMP responses [148]. Additionally, VIP binding is altered with alcohol administration [149, 150]. Taken together, these data suggest that some of the neurological deficits in fetal alcohol syndrome may be related to a reduction of VIP activity in early embryogenesis.

VIP AS A POTENTIAL THERAPEUTIC IN NEURODEVELOPMENTAL DISORDERS

This paper has summarized data that demonstrate the important role of VIP in early postimplantation embryogenesis, especially during the early stages of nervous system development. Whether the neuroregulatory/neuroprotective actions of VIP are direct - as in stimulating mitogenesis, or indirect - by causing the secretion of other neurotrophic/neuroprotective agents, the over or under production of VIP during early embryogenesis could have serious and permanent repercussions on the brain and behavior. The review has included discussion of mouse models of the developmental disorders Down syndrome and fetal alcohol syndrome in which VIP is dysregulated. The possible links between VIP and autism have also been described, as has a deficit in social behavior that is apparent in mice experiencing a blockage of VIP during the period of neural tube closure, the period during which the disordered neural development resulting in autism is thought to occur. The indication of an imbalance of VIP in these several neurodevelopmental disorders not only highlights the importance of VIP during embryogenesis but also suggests that both environmental insults and variant genes can influence the developmental path regulated by VIP. Although the efficacy of countering the under or over production of VIP through pre- or postnatal treatment is the major question, the potential of the neuropeptide VIP as a therapeutic agent in CNS disorders has been given strong consideration.

VIP has been used in a mouse model of excitotoxic white matter lesions where the N-methyl-D-aspartate agonist, ibotenate, mimics the spectrum of neocortical lesions occurring in human newborns, including white matter cysts and decreases in white-matter thickness, such as in periventricular leukomalacia [151]. Perinatal brain injury has been linked to prenatal hypoxia or ischemia, maternal infection, genetic factors or dysregulation of growth factors or proinflammatory cytokines. Survival is often accompanied by neurological impairment such as cerebral palsy; currently there are few therapies to treat perinatal injury. In preterm infants, periventricular leukomalacia is characterized by necrotic and cystic periventricular white matter and is thought to be related to excess excitatory stimulation and reactive oxygen species [152]. Intracerebroventricular co-treatment of VIP with ibotenate provided a dose-dependent decrease in white matter cysts with the highest doses resulting in normal appearing white matter. Co-treatment of ibotenate and a VIP hybrid antagonist resulted in increased lesions suggesting that endogenous VIP plays a role in preventing excitotoxic cortical damage from excitotoxic events [151]. In this model, VIP was not successful when administered intraperitoneally nor did VIP treatment prevent the initial formation of the lesion. VIP appeared to be protective through regrowth and repair mechanisms. The use of the VIP analogs, stearyl-norleucine-VIP (SNV) [153] and RO-25-1553 [154], which are less susceptible to enzymatic degradation, prevented white matter cysts when administered either intraperitoneally or intracerebroventricularly in this model. Furthermore, the intraperitoneal administration of these analogs provided protection up to 12 h after ibotenate treatment [155]. These studies illustrate that VIP or VIP analogs may prove useful in the prevention of perinatal brain injury.

The neuroprotective properties of VIP have made it a potential candidate for the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Mice deficient in apolipoprotien E (Apo E) serve as a model of Alzheimer's disease and treatment of this mouse model with the VIP agonist, SNV, was shown to result in increased cholinergic activity, and prevented the developmental delay and cognitive deficits of the ApoE knockout mice [156]. The prevention of Alzheimer-like symptoms in these mice indicated that VIP and VIP agonists might prove useful in preventing the learning and memory deficits found in many developmental disorders. VIP and SNV have also been shown to protect PC12 cells and neuroblastoma cells from the oxidative stress contributing to the toxicity of dopamine and 6-hydroxydopamine. These peptides may prove useful in protecting neurons in disorders such as Parkinson's disease in which it is hypothesized that dopamine-activated apoptosis may be a causal factor [157].

A short half-life in the body and difficulty crossing the blood brain barrier are two properties of peptides that have

been thought to make them poor candidates as pharmaceuticals for neurological disorders. However, recent work has shown that peripherally administered VIP does reach the brain through a non-saturable mechanism of transmembrane diffusion [158]. This study further indicated that peripherally administered VIP reached the brain with limited enzymatic degradation, illustrating that VIP is in a protected environment in the brain. Intracerebroventricularly administered VIP is not transported out of the brains suggesting that VIP released from cephalic neurons is likely to remain in the brain. Although only a small percentage of peripherally administered VIP reaches the brain, the properties outlined above would allow an increase in brain levels of VIP with peripheral administration, perhaps increasing its usefulness in treating neurological disorders. Other approaches to making peptide pharmaceuticals more efficacious have been to increase the permeability of VIP to cell membranes and to inhibit enzymatic degradation of VIP peripherally with the formation of SNV [153]. This, and similar analogs of VIP, have provided protection in several models of neurodegenerative disease [57].

An additional problem with markedly changing the levels of VIP in the body is that this neuropeptide has such widespread actions; increases or blockage of the peptide might result in undesirable side effects. In the rodent embryo, SHV stimulates embryonic growth through only a subset of VIP receptors [8]. This feature might make this, and other analogs, useful in increasing desirable VIP-dependant actions without altering VIP action throughout the body. A current focus is on the downstream activators of VIP, ADNF and ADNP. The usefulness of active peptides of these highly potent proteins in neurological disease is currently being investigated [57,159].

Future strategies with the aim of increasing the usefulness of VIP as a pharmaceutical could include enhancing the interaction of VIP with biomimetic phospholipid mono and bilayers that could increase its stability and bioactivity [160]. Alternatively, cell or tissue-specific gene delivery of VIP or VIP analogs could bypass the potential problems incurred by administering a peptide that has such diverse and wide spread actions in the body. The addition of VIP to a specific site would prevent stimulation of VIP actions throughout the body. VIP has been shown to induce the differentiation of neurons without affecting the proportion of glial cells in embryonic stem cells [49]. This feature makes the neuropeptide VIP a useful agent in neuronal replacement strategies in neurodegenerative disorders.

The rationale underlying the potential usefulness of VIP in the treatment of neurodevelopmental disorders is based on the important role of VIP during development and the discovery of anomalous VIP in human disorders of this kind. Both VIP and VIP antagonists can reach embryonic and fetal tissues after intraperitoneal administration to pregnant mice [7, 27, 72], demonstrating that prenatal application of these peptides can act upon *in utero* developing tissues. Also, the peri- and postnatal treatment with VIP and VIP analogs in animal models also indicates that these peptides could be useful in the treatment of newborns at risk for neurodevelopmental disorders [151, 153]. The problems inherent in the use of peptides as pharmaceuticals are currently being addressed with modern techniques that not only enhance the stability of these compounds but also limit their actions to specific anatomical sites.

ABBREVIATIONS

ADNF	=	Activity dependent neurotrophic factor
ADNP	=	Activity dependent neuroprotective protein
Apo E	=	Apolipoprotien E
IGF-1	=	Insulin-like growth factor 1
IL	=	Interleukin
NGF	=	Nerve growth factor
PACAP	=	Pituitary adenylate cyclase activating peptide
PHI	=	Peptide histidine isoleucine
SNV	=	Stearyl-norleucine-VIP
VIP	=	Vasoactive intestinal peptide

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