Recent advances in vip physiology and pathophysiology

Vasoactive intestinal peptide (VIP) is a 28-residue amino acid peptide initially isolated from porcine duodenum and first characterized in 1970 [1]. A member of the secretin/glucagon hormone superfamily [1, 2], VIP is evolutionarily well conserved with sequence homology among fish, frogs, and humans [3]; for mammals, except for guinea pigs and chickens [4], the sequence homology is at least 85% [5]. Although VIP was discovered as a potent vasodilator, VIP was subsequently found to be widely distributed in the central and peripheral nervous system as well as in the digestive, respiratory, reproductive, and cardiovascular systems as a neurotransmitter and neuroendocrine releasing factor [5-6], contributing to a wide range of physiological and pathological processes related to development, growth, and the control of neuronal, epithelial, and endocrine cells. VIP has also been implicated in the regulation of carcinogenesis, immune responses, and circadian rhythms [7].

Historical Background

In the late 1960s, Dr. Sami I. Said reported that injection of extracts of mammalian lungs produced systemic vasodilation and hypotension. Together with Dr. Viktor Mutt, Dr. Said turned his search from the lung to duodenal extracts, which were more readily available, based on the premise that the same peptide might be present in other organs. They soon discovered that peptide fractions from porcine duodenum indeed contained a vasodilator component, which supported Bayliss and Starling’s assumption (made in 1990 during their discovery of secretin) that a ‘ vasodepressor principle’ was present in intestinal extracts [8].

A few years later, VIP was identified in the central and peripheral nervous systems [9] and has since been recognized as a widely distributed neuropeptide, acting as a neurotransmitter or neuromodulator in many organs and tissues, including heart, lung, thyroid gland, kidney, immune system, urinary tract, and the genital organs [3]. The widespread distribution of VIP is correlated with its involvement in an extensive variety of biological activities, including systemic vasodilation, increased cardiac output, bronchodilation, hyperglycemia, smooth muscle relaxation, promotion of growth, hormonal regulation, analgesia, hyperthermia, neurotrophic effects, learning and behavior, bone metabolism, and some differential effects on gastrointestinal secretion and on gastric motility [10].

Structure and Classification

The three-dimensional structure of VIP is similar to other members of the glucagon and secretin family [2], in which the structure, function, and signaling activity of pituitary adenylyl cyclase-activating peptide (PACAP), isolated in 1989, is the most closely related peptide to VIP and they share 68% sequence homology with each other [10].

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VIP is synthesized from a precursor molecule, prepro-VIP, located in the chromosomal region 6q24 that is approximately 9 kb in length containing seven exons [6], each encoding a functional domain. The 170-amino acid prepro-VIP is metabolized by a signal peptidase in the endoplasmic reticulum to yield the 148-amino acid precursor peptide termed pro-VIP, which is then cleaved by prohormone convertases to a form of VIP containing the internal

cleave-amidation site GKR (VIP-GKR; prepro-VIP $_{125\text{-}155}$)[11]. The KR residues of VIP-GKR are then cleaved by carboxypeptidase-B-like enzymes to VIP-G [12], which then can be metabolized by peptidyl-glycine alpha-amidating monooxygenase (PAM) enzymes to VIP, which has an amidated C terminus [10]. [do not exactly copy published description, add your own explanations etc.]

VIP vary its conformation depending on the environment. Most notably, the forms of an α-helix are induced by an anionic lipid bilayer or liposome when they bind to the receptors [5].

Receptors

The two receptors that recognize VIP termed VPAC1 and VPAC2 are class B G protein-coupled receptors (GPCRs) that include PAC1, secretin, glucagon, glucagon-like peptide (GLP)-1 and -2, calcitonin, gastric inhibitory peptide (GIP), corticotropin-releasing factor (CRF)-1 and -2, and parathyroid hormone (PTH) receptors. VPAC1 and VPAC2 are activated by PACAP as well as by VIP, even though PACAP has its own specific receptor, PAC1, for which VIP has very low affinity [13]. Through these receptors, VIP can mediate an extensive number of functions such as regulating gastric acid and intestinal secretions, enzyme release from the exocrine and endocrine pancreas, cellular motility, vasodilation, and intestinal contractility [14–16]. VPAC1, for which no receptor splice variants known, was first isolated and identified from the rat lung and later identified in human tissues. Selective agonists and antagonists have been synthesized for anticipated experimental and clinical use [17, 18].

The abundance and localization of VPAC1 in the colon of mice and humans may indicate that some of the key functions of VIP, such as increasing the rate of epithelial regeneration and ion transport, fluid secretion, mucus secretion, and affecting tight junction protein expression can be directly mediated through epithelial luminal receptors [19]. Most commonly occurring human tumors express VPAC1 receptors although tumors predominantly expressing VPAC2 are rare. VPAC2 receptors are predominantly expressed in smooth muscle throughout the GI tract except for the colon [20]. VPAC2 is also expressed at a high level in pancreatic cells. VPAC2 receptor knockout mice displayed growth retardation, decreased fat mass, and increased lean mass, and reduced serum leptin level with increased basal metabolic rate [21].

Functions in the gastrointestinal tract

VIP and its receptors are expressed broadly in the gastrointestinal tract. The majority of VIP actions are mediated through the VCAP1 receptor expressed on apical membranes of the mucosal, cholinergic excitatory motor neurons innervating longitudinal muscles, cholinergic secretomotor neurons, and mucosal mast cells [22]. VPAC1 is expressed by somatostatin containing gastric D cells; their activation is believed to inhibit gastric acid secretion [14]. VIP regulates gastrointestinal (GI) motility, vasodilation, sphincter relaxation, water absorption, iron uptake, mucus secretion, and immune homeostasis [23]. VIP also affects numerous pathological conditions affecting the human gut, including VIP-secreting pancreatic and neural crest tumors (VIPomas), insulin-dependent diabetes, Hirschsprung’s disease, and inflammatory bowel syndromes such as Crohn’s disease and ulcerative colitis

[24]. VIP affects epithelial cyclic AMP metabolism, transcellular Na and Cl fluxes, and short-circuit current in rabbit ileum, similar to those observed in response to the application of pro-secretory bioactive compounds such as cholera enterotoxin and certain prostaglandins. VIP is the only one of 10 potential secretagogues to increase cyclic AMP levels in ileal epithelial cells [25]. Similar to glucagon, VIP stimulates hepatic glycogenolysis and myocardial contraction; like secretin, it stimulates pancreatic exocrine secretion; and like both glucagon and secretin, VIP stimulates lipolysis [25]. VIP also influences epithelial barrier function, with consequent effects on inflammation susceptibility. VIP-ergic neurons in the submucosa directly innervate intestinal epithelial crypt cells, augmenting intestinal ion and fluid secretion via VPAC1 and cAMP [26].

VIP indirectly modulates epithelial permeability via regulation of the expression and function of epithelial tight junction proteins. VIP-ergic pathways increase expression of the tight junction protein zona occludens in the human submucosal neuronal layer and reduce paracellular epithelial permeability [27].

VIPomas, the best-characterized association of VIP with a tumor, is also known as pancreatic cholera or the Verner-Morrison syndrome. Hypersecretion of VIP by this ectopic tumor causes large-volume, watery diarrhea, hypokalemia, and hypochlorhydria [28], due to the action of VIP on VPAC1 receptors in the intestinal mucosa that increases chloride water movement into the intestinal lumen [29].

VIP and irritable bowel syndrome (IBS)

Irritable bowel syndrome (IBS) with diarrhea (IBS-D) correlates with mast cell function and VIP release. Mast cells are activated, leading to increased plasma concentrations of substance P & VIP in IBS-D patients, especially in women [30]. VIP indirectly modulates epithelial permeability via regulation of expression of epithelial tight junction proteins [27]. In contrast, female IBS-D patients have higher plasma VIP and tryptase concentrations, accompanied by a greater overall number with a higher percentage of VPAC1-positive mast cells in colonic biopsies compared to data from controls. Furthermore, higher transcellular bacterial passage through the colonic biopsy, inhibitable with anti-VPAC antibodies or a mast cell stabilizer ketotifen have been observed [31], suggesting that mast cells and VIP are key modifiers of bacterial translocation in the colonic mucosa of IBS-D patients.

VIP and Colitis

The connection of VIP with inflammatory bowel disease (IBD) and colitis has yet to be fully elucidated; the contribution of VIP towards the pathogenesis of dextran sulfate sodium (DSS) and 2, 4, 6-trinitrobenzene sulfonic acid (TNBS) induced models of colitis in mice is controversial [32]. VIP was proposed as a biomarker for IBD in a study reporting elevated VIP plasma concentrations during active disease phases [33]; several studies described an anti-inflammatory role of VIP in *vitro* and in mice; although administration of the peptide itself in mouse models of colitis improves the clinical score and gastrointestinal inflammation, other groups reported that VIP administration by constant infusion enhanced the severity of colitis [34]. VIP knockout mice are reported to exhibit abnormalities of the gastrointestinal tract in some

cases [24]. Most recently, the recombinant VIP analog (rVIPa) was reported to ameliorate TNBS-induced colonic injury and inflammation, effectively preserving intestinal mucosal barrier function in rats [35]. These discordant findings provide tantalizing suggestions that VIP or its related analogs may have therapeutic promise in IBD and colitis patients.

VIP and Immunity

In the colon, VIP is pro-secretory and is also anti-inflammatory. VIP downregulates the abundance of pro-inflammatory cytokines and mediators such as interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-12, nitric oxide, and chemokines [36]. VIP, also produced by type 2 T helper lymphocytes (Th2), could also be classified as a Th2 cytokine [10, 36, 37]. The potent anti-inflammatory effects of VIP may result from its promotion of Th cell differentiation toward a “ Th2” phenotype [10]. Moreover, VIP also increases regulatory T cell production while inhibiting macrophage pro-inflammatory actions, all contributing to its anti-inflammatory effects. VIP usually increases the rate of epithelial ion secretion via activation of VPAC1 that increases cAMP production. Yet, VIP can also have antisecretory effects through the inhibition of cyclooxygenase-2 (COX-2) and nuclear factor (NF)-κB. Furthermore, VIP reduces paracellular permeability via regulation of the expression and function of tight junction proteins [36].

VIP maintains immunological tolerance and homeostasis in the gut primarily by regulation of T cell responses and Toll-like receptor (TLR)-mediated innate immune responses. The effects of VIP are mediated by VPAC1 and VPAC2 expressed on T cells; VPAC1 is constitutively expressed on T cells whereas VPAC2 expression is induced by inflammation [37]. VIP promotes Th2-like

responses and inhibits Th1 immune responses via VPAC2 as VPAC2 gene depletion increases Th1-type responses [38].

Therapeutic Potential of VIP

Although VIP has well-studied anti-inflammatory and other therapeutic potentials, it has not been successful to design VIP-based drug due to the intrinsic instability of the peptide that limits bioavailability and delivery. A major drawback to the clinical utility of VIP is its high sensitivity to proteolytic [39]. Another limiting factor is the existence of multiple cellular targets that bind VIP at high affinity. Hence, the targeted delivery of the VIP or its analogs via nanoparticles is a desirable option [36]. Recent advances in the field include synthesis of stable analogs such as lipophilic or peptide derivatives of VIP that mimic the activity of native VIP [36]. Nanobodies are single-domain antibody fragments derived from heavy chain-only antibodies of *Camelidae* that function like conventional antibodies but possess numerous advantages in challenging environments where conventional antibodies often fail. The newly generated and characterized nanobodies recognizing human VPAC1 should thus be useful tools for further investigation of VIP function [39].

Summary and Conclusions

Since its discovery in 1970, VIP has been studied in various systems in the body including the gastrointestinal, respiratory, cardiovascular, immune, central and peripheral nervous system, and found to play various key roles. However, due to the abundance and multiple roles in the system, it has been challenging to extrapolate the precise involvement within the system.

Specifically, in the gut, VIP has shown therapeutic potential for a variety of inflammatory disorders such as IBD and colitis. The recent discoveries of nanobodies are expected to add preferably to the potential of potential drug availabilities. In order to make use of VIP as a therapeutic measure, it is essential to further study its localization and point of action. References

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