Vasoactive intestinal peptide: cardiovascular effects

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Abstract

Vasoactive intestinal peptide (VIP) is present in the peripheral and the central nervous systems where it functions as a nonadrenergic, noncholinergic neurotransmitter or neuromodulator. Significant concentrations of VIP are present in the gastrointestinal tract, heart, lungs, thyroid, kidney, urinary bladder, genital organs and the brain. On a molar basis, VIP is 50–100 times more potent than acetylcholine as a vasodilator. VIP release in the body is stimulated by high frequency (10–20 Hz) nerve stimulation and by cholinergic agonists, serotonin, dopaminergic agonists, prostaglandins (PGE, PGD), and nerve growth factor. The VIP peptide combines with its receptor and dose-dependently activates adenylyl cyclase. The vasodilatory effect of VIP in different vascular tissues or species also may be due to increases in nitric oxide, cyclic GMP, and other signaling agents. In the heart, VIP immunoreactive nerve fibers are present not only in the epicardial coronary arteries and veins, but also the sinoatrial node, atrium, interatrial septum, atrioventricular node, intracardiac ganglia, and ventricles (right ventricle > left ventricle). In the coronary arterial walls, VIP may contribute to the regulation of normal coronary vasomotor tone. In research animals and in humans, VIP, administered into the coronary artery or intravenously, increases the epicardial coronary artery cross-sectional area, decreases coronary vascular resistance, and significantly increases coronary artery blood flow. High frequency parasympathetic (vagal) nerve stimulation also releases endogenous VIP in the coronary vessels and heart and significantly increases coronary artery blood flow. In addition, the release of VIP in the heart is increased during coronary artery occlusion and during reperfusion where VIP may promote local blood flow and may have a free-radical scavenging effect. VIP also has a primary positive inotropic effect on cardiac muscle that is enhanced by its ability to facilitate ventricular–vascular coupling by reducing mean arterial pressure by 10–15%. In concentrations of 10^{-9} – 10^{-7} mol, VIP augments developed isometric force and increases atrial and ventricular contractility. The presence of VIP-immunoreactive nerve fibers in and around the sinus and the atrioventricular nodes of mammals strongly suggests that this peptide can affect the heart rate. In this regard, endogenously released or exogenous VIP can significantly increase the heart rate and has a more potent effect on heart rate than does norepinephrine. The presence and significant cardiovascular effects of VIP in the heart suggests that this peptide is important in the regulation of coronary blood flow, cardiac contraction, and heart rate. Current investigations are defining the physiological role of VIP in the regulation of cardiovascular function. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Contractile function; Coronary circulation; Heart rate (variability); Neurotransmitters; Vasoconstriction/dilation

1. Introduction/General considerations

1.1. Discovery and localization

Said and Mutt first isolated vasoactive intestinal peptide (VIP) from the porcine duodenum in 1970 [1]. The name VIP is derived from the profound and long-lasting gastrointestinal smooth muscle relaxation that this peptide produces following systemic administration [2]. Mutt and Said established the amino acid sequence of VIP in 1973 and this work enabled the synthesis of the VIP peptide shortly thereafter [3,4]. VIP contains 28 amino acid residues with a molecular weight of 3326 (Table 1). The primary structure of VIP is closely related to pituitary adenylate cyclase activating polypeptide (PACAP) and, to a much lesser extent, to secretin, glucagon, gastric inhibitor polypeptide and helodermin-like peptides [5]. The amino acid sequence of VIP in man, cow, sheep, goat, dog,
Table 1
Amino acid sequence of VIP and related peptides

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIP</td>
<td>H</td>
<td>S</td>
<td>D</td>
<td>A</td>
<td>V</td>
<td>F</td>
</tr>
<tr>
<td>VIP (gp)</td>
<td>H</td>
<td>S</td>
<td>D</td>
<td>A</td>
<td>L</td>
<td>F</td>
</tr>
<tr>
<td>PACAP 27</td>
<td>H</td>
<td>S</td>
<td>D</td>
<td>G</td>
<td>L</td>
<td>F</td>
</tr>
<tr>
<td>PHI</td>
<td>H</td>
<td>A</td>
<td>D</td>
<td>G</td>
<td>V</td>
<td>F</td>
</tr>
<tr>
<td>Secretin</td>
<td>H</td>
<td>S</td>
<td>D</td>
<td>G</td>
<td>T</td>
<td>F</td>
</tr>
<tr>
<td>Glucagon</td>
<td>H</td>
<td>S</td>
<td>Q</td>
<td>G</td>
<td>T</td>
<td>F</td>
</tr>
</tbody>
</table>

* PACAP27 has 70% homology with VIP, PHI has 48% homology, secretin has 33% homology and glucagon has 20% homology with VIP. The VIP sequence is identical in human, rat, rabbit, dog, sheep, goat and cow but differs in four amino acid substitutions in the guinea pig (gp). The one-letter codes for the amino acids are: Ala, A; Cys, C; His, H; Met, M; Thr, T; Arg, R; Glu, E; Ile, I; Phe, F; Trp, W; Asn, N; Gln, Q; Leu, L; Pro, P; Tyr, Y; Asp, D; Gly, G; Lys, K; Ser, S; Val, V.

rabbit and rat is identical to that of the porcine peptide [6]. Guinea pig VIP and non-mammalian VIP (chicken, alligator, frog, trout, bowfin, dogfish, cod, and goldfish) differ from the human sequence at only four or five positions [6]. The VIP gene has been cloned, sequenced, and localized to chromosome 6q24 [7–9].

The VIP peptide is derived from prepro-VIP, which consists of 170 amino acid residues [6]. Proteolytic cleavage of prepro VIP yields: prepro VIP(22–79), peptide histidine isoleucine/methionine (PHI/PHM)(81–107), prepro VIP(111–122), VIP(125–152), and prepro VIP(156–170) [6] (Fig. 1). Peptide histidine isoleucine/methionine is structurally related to VIP and shares many of VIP's biological actions but is generally less potent than VIP. Other peptide sequences have been identified during the proteolytic cleavage of the VIP precursor but have limited biological effects. Nevertheless, the bioactivity of these partial sequences of VIP increases with increasing amino acid chain length [10].

Early investigators hypothesized that VIP occurred in ‘endocrine-like’ cells in the gastrointestinal tract. Subsequently VIP was discovered in nerve cell bodies and axons in the gastrointestinal wall and in the peripheral and central nervous systems where the peptide functions as a nonadrenergic, noncholinergic neuropeptide transmitter or neuromodulator. Immunofluorescent and radioligand assays have localized VIP to neuronal cell bodies, axons and dendrites, and presynaptic nerve terminals from which VIP is released as a neurotransmitter [11]. In the peripheral nervous system, VIP is present in sympathetic ganglia, the vagus nerves, some motor nerves such as the sciatic nerve, autonomic nerves that supply exocrine glands, vascular and nonvascular smooth muscle, and ganglion-like clusters of neuronal cell bodies that provide ‘intrinsic’ organ innervation [11,12]. The gastrointestinal tract, heart, lungs, thyroid, kidney, urinary bladder, and genital organs contain significant concentrations of VIP. In the central nervous system, VIP is present in the cerebral cortex, the hypothalamus, amygdala, hippocampus, corpus striatum, and the vagal centers of the medulla oblongata [6,13,14].

In the peripheral nervous system, VIP containing neurons are either intrinsic neurons involved in local reflexes, or postganglionic neurons under preganglionic cholinergic (nicotinic) control [15]. In postganglionic neurons, VIP is synthesized in neuronal cell bodies and is then exported along axons or dendrites to large 100 nm spherical dense core vesicles located in presynaptic nerve terminals [16,17]. The transport rate of VIP to nerve terminals is approximately 9 mm/h [17]. Many systemic blood vessels and also pulmonary blood vessels are innervated by VIP immunoreactive nerve fibers, which cause vascular smooth muscle dilation [15]. In this regard, VIP, on a molar basis, is 50 to 100 times more potent than acetylcholine as a vasodilator [11]. In addition, VIP facilitates the secretory response to acetylcholine in glandular epithelium and is involved in the control of exocrine as well as endocrine secretion, not only in the gastrointestinal tract but also in the respiratory and urogenital tracts [15,18,19]. Endogenous VIP is released by high frequency nerve stimulation [17] and also is released by neostigmine, as well as by serotonin, dopaminergic agonists such as bromocriptime.
and apomorphine, prostaglandins (PGE, PGD) and nerve growth factor [20].

In the central nervous system, VIP contributes to the regulation of cerebral blood flow, energy metabolism and enzymatic activity, and is twenty times more potent than norepinephrine in stimulating the enzymatic breakdown of glycogen to glucose [6,13,14]. VIP is also involved in the release of corticotropin-releasing hormone, prolactin, oxytocin, and vasopressin [21].

1.2. VIP receptors

The VIP receptor is a member of a family of guanine nucleotide binding protein (G protein)-coupled receptors which include receptors for pituitary adenylate cyclase activating polypeptide (PACAP), secretin, glucagon, calcitonin, parathyroid hormone, growth hormone-releasing factor (GHRF) and corticotropin-releasing factor [22,23]. VIP receptors are present in the heart [24] and blood vessels [25,26], as well as in many other tissues, and have a molecular weight of 43 000–80 000 daltons. This variability in molecular weight is consistent with either cell-specific differential glycosylation of the same receptor or receptor heterogeneity. Two subtypes of VIP receptors, VPAC1 and VPAC2, have been cloned from rat and human tissue and sequenced, and there is 50% identity between the two receptor subtypes [27–29]. These receptors are identical to the PACAP type 2 and 3 receptors. The two subtypes of the VIP receptor share characteristics also seen in other members of the secretin receptor family, including an extensive amino-terminal signal peptide recognition sequence, several extracellular glycosylation sites and cysteine residues, seven transmembrane-spanning domains and a number of intracellular sites for phosphorylation by protein kinase C [27–29]. VPAC1 receptors are widely distributed in the central nervous system (cerebral cortex, amygdaloid nuclei, hippocampus), and in the liver, lung, intestine and are also present in aorta, heart, adipose tissue, and blood vessels of the pancreas, intestine, and heart [6,14,27,29,30]. VPAC2 receptors are abundant in the olfactory lobes, thalamus, and the supraschiasmatic nucleus, and are present in lower concentrations in hippocampus, brainstem, spinal cord and dorsal root ganglia [6,14,29,31]. VPAC2 receptors are also present in the aortic endothelium, heart, pancreas, renal medulla, adrenal cortex, skeletal muscle, and adipose tissue [14,28–31]. Investigations are focused at the present time on developing specific agonists and antagonists for the VIP receptor subtypes. Currently available are specific VPAC1 receptor agonists ([Arg16]chicken secretin and [Lys15, Arg16, Leu27]VIP (1–7)GRF (8–27)–NH2), VPAC2 receptor agonists (Ro 25–1553 and Ro 25–1392), and a VPAC1 receptor antagonist ([Ac–His1, β-Phe2, Lys3, Arg5]VIP(3–7)GRF(8–27)–NH2) [23].

VIP combines with its receptor and dose-dependently activates adenylyl cyclase, as demonstrated in cerebral vessels [32,33], aorta [34–36], heart and coronary vessels [37–40], the mesenteric artery [41,42], portal vein [43] and ovarian artery [44] (Table 2). The degree of VIP-induced activation of adenylyl cyclase varies with the species (rat, rabbit, dog, cat, monkey) and also the organ or tissue [45,46]. In the rat, VIP increases adenylyl cyclase activity in a dose and GTP-dependent fashion with an EC50 value that varies with the tissue from 10−8 to 10−6 M [32,37,41] (Table 2). The dose-dependent increase in adenylyl cyclase activity and cAMP concentration corresponds well with VIP’s ability to produce vasodilation in isolated arteries [41]. In cerebral microvessels, the effects of VIP on adenylyl cyclase activity are additive with the effects of isoproterenol, 2-chloroadenosine, and prostaglandin E1 [32]. This suggests compartmentalization of the effects of VIP on adenylyl cyclase activity, possibly involving different receptors, G proteins, and adenylyl cyclase isozymes. In the rat mesenteric artery, VIP is 100-fold more potent than isoproterenol and prostaglandin E1 in enhancing adenylyl cyclase activity [41]. Moreover, secretin, gastric inhibitory peptide, glucagon, angiotensin II, or substance P do not alter VIP’s activation of adenylyl cyclase in the portal vein [43].

In vascular smooth muscle, a VIP-induced increase in cAMP concentration can activate protein kinase A, which phosphorylates phospholamban, and thereby increases the sequestration of Ca2+ by the sarcoplasmic reticulum [47]. Cyclic AMP can also increase the activity of the sarcosomal Ca2+ pump ATPase, thereby increasing the extrusion of Ca2+ into the extracellular space. In addition, cAMP decreases the affinity of myosin light chain kinase for the Ca2+-calmodulin complex, thereby reducing myosin phosphorylation and decreasing actin–myosin affinity [47]. These processes when activated by VIP can produce smooth muscle relaxation and vasodilation.

The vasodilatory effect of VIP in different vascular tissues or species is not solely due to an increase in cyclic AMP. The vasorelaxant effect of VIP is dependent on the endothelium in the rat aorta, the bovine intrapulmonary artery, and the human uterine artery, and is mediated by activation of lipoxgenase in the rat aorta and by nitric oxide and activation of guanylyl cyclase in the human uterine artery [48–50]. In the bovine intrapulmonary artery, endothelial-dependent vasorelaxation in response to VIP involves activation of guanylyl cyclase and cyclooxygenase through two pathways, which are probably mediated by nitric oxide and prostacyclin [49]. In this system, cyclic AMP and cyclic GMP may interact synergistically to initiate and sustain the vasodilatory response to VIP [51]. The vasorelaxant effect of VIP is independent of the endothelium in the feline middle cerebral artery [33,32], canine carotid artery [53], canine hepatic artery [54], porcine coronary artery [55] and the rat portal vein [43]. Moreover, the vasorelaxant effect of VIP in some species may ultimately involve hyperpolarization of the vascular smooth muscle membrane, which reduces calcium
Table 2
Effect of VIP on adenylyl cyclase activity (AC activity), cyclic AMP content (cA content) or cyclic GMP content (cG content) in various tissues, after indicated incubation times (Incubation), showing effective dose range, EC\(_{50}\) and maximal effect*.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Parameter</th>
<th>Incubation time (min)</th>
<th>Effective dose range (M)</th>
<th>EC(_{50}) (M)</th>
<th>Max. effect (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood vessel</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCeMV</td>
<td>AC activity</td>
<td>10^{-8}−10^{-6}</td>
<td>10^{-7}</td>
<td>95</td>
<td></td>
<td>[32]</td>
</tr>
<tr>
<td>RPVM(ed)</td>
<td>AC activity</td>
<td>10^{-8}−10^{-5}</td>
<td>10^{-7}</td>
<td>575</td>
<td></td>
<td>[43]</td>
</tr>
<tr>
<td>RMSMC</td>
<td>AC activity</td>
<td>10^{-8}−10^{-6}</td>
<td>10^{-8}</td>
<td>167</td>
<td></td>
<td>[41]</td>
</tr>
<tr>
<td>RAoSMC</td>
<td>AC activity</td>
<td>10^{-8}−10^{-7}</td>
<td>10^{-9}</td>
<td>233</td>
<td></td>
<td>[41]</td>
</tr>
<tr>
<td>RMSMC</td>
<td>cA content</td>
<td>1</td>
<td>10^{-9}−10^{-6}</td>
<td>10^{-7}</td>
<td>712</td>
<td>[41]</td>
</tr>
<tr>
<td>BPAR</td>
<td>cA content</td>
<td>1</td>
<td>10^{-9}−(3×10^{-4})</td>
<td>ND</td>
<td>(340)</td>
<td>[49]</td>
</tr>
<tr>
<td>RBMSM</td>
<td>cA content</td>
<td>3</td>
<td>10^{-9}−(1×10^{-7})</td>
<td>ND</td>
<td>(88)</td>
<td>[42]</td>
</tr>
<tr>
<td>RBaO</td>
<td>cA content</td>
<td>10</td>
<td>10^{-9}−10^{-7}</td>
<td>10^{-8}</td>
<td>750</td>
<td>[44]</td>
</tr>
<tr>
<td>RAoSMC</td>
<td>cA content</td>
<td>10</td>
<td>10^{-9}−(3×10^{-7})</td>
<td>ND</td>
<td>(593)</td>
<td>[34]</td>
</tr>
<tr>
<td>RAoR(ed)</td>
<td>cA content</td>
<td>10</td>
<td>10^{-9}−10^{-6}</td>
<td>10^{-7}</td>
<td>670</td>
<td>[35]</td>
</tr>
<tr>
<td>BPAR</td>
<td>cG content</td>
<td>1</td>
<td>10^{-9}−(3×10^{-5})</td>
<td>ND</td>
<td>(400)</td>
<td>[49]</td>
</tr>
<tr>
<td><strong>Heart</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCM</td>
<td>AC activity</td>
<td>10^{-8}−3×10^{-6}</td>
<td>10^{-6}</td>
<td>150,42</td>
<td></td>
<td>[37,45]</td>
</tr>
<tr>
<td>DLVM</td>
<td>AC activity</td>
<td>10^{-9}−3×10^{-6}</td>
<td>3×10^{-7}</td>
<td>75</td>
<td></td>
<td>[45]</td>
</tr>
<tr>
<td>MLVM</td>
<td>AC activity</td>
<td>10^{-9}−(1×10^{-3})</td>
<td>ND</td>
<td>(427)</td>
<td></td>
<td>[45]</td>
</tr>
<tr>
<td>RVMMyo</td>
<td>cA content</td>
<td>5</td>
<td>10^{-9}−10^{-8}</td>
<td>5.8×10^{-4}</td>
<td>60</td>
<td>[39]</td>
</tr>
<tr>
<td>RNCMyo</td>
<td>cA content</td>
<td>15</td>
<td>10^{-9}−10^{-7}</td>
<td>10^{-8}</td>
<td>500</td>
<td>[40]</td>
</tr>
<tr>
<td>RNCMyo</td>
<td>cA content</td>
<td>60</td>
<td>10^{-10}−(1×10^{-8})</td>
<td>ND</td>
<td>(161)</td>
<td>[40]</td>
</tr>
</tbody>
</table>

* If maximal effect was not determined, then maximal dose used and its effect are shown in parentheses. ND indicates that EC\(_{50}\) was not determined.

Influx and the intracellular calcium concentration [42.56–58].

The precise contributions of cyclic AMP, cyclic GMP, nitric oxide and other signaling agents to the vasodilation elicited by VIP in different vascular beds is not known. One possible mechanism for the interaction between these mediators of vasodilation is present in gastrointestinal smooth muscle, in which VIP elicits relaxation via activation of both cyclic AMP and cyclic GMP-dependent pathways [59,60] (Fig. 2). The increase in cyclic AMP is due to activation of VPAC receptors [61], whereas the increase in cyclic GMP may be due to activation of natriuretic peptide clearance receptors (NPR-C) coupled to a membrane-bound endothelial nitric oxide synthase [62]. Whether a similar interaction exists in vascular smooth muscle or in the heart is presently known and is under investigation.

After VIP binds to its receptor, the peptide is rapidly internalized, probably by receptor mediated endocytosis [63]. This internalization decreases the cell surface receptor density. Most of the receptors are recycled back to the cell surface, but some receptors are degraded in lysosomes [63]. The complete cycle of internalization and recovery of the receptor requires a half-time of 13 min [63].

In ventricular myocytes, a VIP-induced increase in cyclic AMP can increase protein kinase A activity, which enhances calcium channel phosphorylation, the L-type calcium current, and the release of calcium from the sarcoplasmic reticulum (Fig. 2). As a consequence, the intracellular calcium concentration increases, enhancing cardiac myocyte tension development and the rate and extent of contraction [64]. An increase in cyclic AMP can also increase troponin I and phospholamban phosphorylation, which decreases the affinity of troponin for calcium, enhances intracellular calcium sequestration, and subsequently enhances the rate and extent of myocyte relaxation [64]. In this manner, VIP can increase cardiac myocyte contraction and relaxation. These effects of VIP on cyclic AMP and L-type calcium current are significantly decreased by VIP antagonists, including VIP(7–28)/neurotensin or [N-acetyl-Tyr-d-Phe2]GRF [40,65].

In the sinoatrial node, a VIP-induced increase in cyclic AMP can activate the hyperpolarization-activated pacemaker current and accelerate the rate of diastolic depolarization and increase the heart rate [66,67]. The VIP effect on the I\(_{f}\) current is reversed by the VIP antagonist [4Cl-d-Phe\(^{6}\).Leu\(^{17}\)]VIP [66,67].

Hypertension, obesity, diabetes, and hypothyroidism reduce the adenylyl cyclase response to VIP in cardiac myocytes [68–70]. In the spontaneously hypertensive rat, the activation of adenylyl cyclase in response to VIP is impaired by as much as 69% [68,69]. In contrast, the activation of adenylyl cyclase in response to the stable guanosine 5’-triphosphate (GTP) analogue, Gpp(NH)p, or sodium fluoride (NaF) is not altered. Moreover, the EC\(_{50}\)
values for the effects of VIP on adenylyl cyclase activity remain unaltered, indicating that the receptor affinity is not impaired. Instead, there appears to be a specific decrease in the density of the VIP receptors or the coupling of the receptors to adenylyl cyclase in hypertensive rats [68,69].

1.3. Plasma concentrations of VIP

VIP that circulates in the plasma of normal individuals originates from VIP-containing nerve fibers in the gastrointestinal tract and also reflects peptide overflow from vascular nerves [71–73]. The half-life of the peptide in the plasma is 48 s [73,74]. The fasting concentration of VIP in human plasma is approximately $2 \times 10^{-12}$ M but may rise to $4.5 \times 10^{-12}$ M with gastrointestinal stimulation [72,74]. Although the concentration of this neuropeptide in the plasma is small, VIP can be released in the tissues and can produce a physiologic effect without significantly increasing the plasma concentration [73,75]. For example, the tissue concentrations of VIP may be as high as 65 ng/g tissue ($=2.0 \times 10^{-8}$ M) in the frontal cortex and hypothalamus [76].

VIP undergoes a circadian rhythm with peak concentrations occurring in the plasma at 1800 h in the elderly and at 2000 h in young individuals [71,72]. During strenuous exercise, plasma VIP concentrations can increase by as much as 100% and persist for more than 20 min after the termination of exercise [72]. This VIP response to exercise is significantly decreased by a glucose infusion, which suggests that VIP has an energy mobilizing function during exercise [72]. In patients with gastrointestinal VIP tumors, which produce a watery-diarrhea syndrome, the plasma concentration of VIP can increase to $4 \times 10^{-10}$ M [77].

The major sites of VIP metabolism are the lungs, the liver, and the kidneys. Metabolism of VIP does not occur in the heart as the peptide is removed by cardiac lymphatic drainage and coronary venous drainage [78,79].

2. VIP physiological actions in the heart

In the heart, VIP immunoreactive nerve fibers are present in the epicardial coronary arteries and veins, the sinoatrial node, atrium, interatrial septum, atrioventricular node, intracardiac ganglia, and ventricles (right ventricle $\gg$ left ventricle) [80]. VIP fibers in the heart arise from postganglionic parasympathetic (vagal) neurons and also intrinsic nerve fibers [80,81].

2.1. Coronary artery effects of VIP

VIP is present in the proximal coronary arterial walls in concentrations of $1.2–2.2 \times 10^{-12}$ mol/g tissue ($=1.2–2.1 \times 10^{-9}$ M) and may contribute to the regulation of normal coronary vasomotor tone [79,82]. Conversely, decreases in the VIP concentration in the coronary arteries may contribute to coronary spasm [82]. The effects of VIP on the coronary arteries have been studied in isolated vascular tissue, in intact hearts and animals, and in patients. In each of these studies, VIP produces significant coronary dilation. The vasodilatory effects of VIP on arteries are much greater than on veins because of the greater VIP receptor density in arterial vessels [83].

In contracted isolated coronary vascular strips, VIP, in concentrations of approximately $1–3 \times 10^{-9}$ M, decreases vascular tension by as much as 94% of the control tension [55,84] and dilates isolated epicardial coronary arteries by 23–43% [85–87]. In animals and also in humans, VIP, in
concentrations of $3 \times 10^{-10} - 3 \times 10^{-9}$ mol intraarterially, increases the epicardial coronary artery cross-sectional area by 27%, decreases coronary vascular resistance by 46%, and increases coronary artery blood flow by 200% [82,88]. This significant decrease in coronary vascular resistance and increase in coronary blood flow suggests that VIP can act on the coronary microcirculation. The coronary dilator effect of VIP, at maximal doses, is significantly greater than that of isoproterenol [89].

In healthy dogs and in dogs with cobalt-induced cardiomyopathy, VIP, in doses of $0.36-0.90 \times 10^{-12}$ mol/kg/h i.v. increases blood flow to the atria and the ventricles by as much as 75% [84]. At doses of $1.8 \times 10^{-9}$ mol/kg/h i.v. in normal dogs, VIP increases the coronary blood flow by 77%, the cardiac index by 55%, but also increases myocardial oxygen consumption by 40% [84]. At the same time, the percent oxygen extracted from the coronary blood by the myocardium decreases as the transcoronary sinus oxygen difference declines by 16% [84]. These results suggest that VIP can increase coronary blood flow in excess of an increase in myocardial oxygen requirements.

VIP administered to patients produces similar hemodynamic effects on coronary blood flow. The effect of VIP on myocardial oxygen requirements is directly dependent on the dose of VIP and the method of administration. When VIP, in doses of $3-90 \times 10^{-12}$ mol/min, is infused directly into the left coronary artery of patients, the major determinants of myocardial oxygen consumption (preload, afterload, contractility, and heart rate) do not change [88]. Moreover, the myocardial extraction of oxygen as measured by the transcoronary sinus $O_2$ content difference decreases progressively in comparison with the baseline extraction [88]. When VIP is infused intravenously, in doses of $0.5-2.0 \times 10^{-10}$ mol/kg/h, the coronary vascular resistance decreases by 33% and the systemic and the pulmonary vascular resistances decrease by 31 and 24%, respectively. However, the myocardial oxygen consumption also increases by 18–25% [90]. These results suggest that VIP causes significant direct coronary artery dilation when administered intracoronary or intravenously but that intravenous VIP can also cause indirect coronary vasodilation by increasing myocardial oxygen requirements. The VIP-induced coronary vasodilation is not mediated by prostaglandins or other cyclooxygenase products, since the coronary sinus concentration of 6-keto-prostaglandin F1α does not increase during the infusion of VIP and cyclooxygenase inhibition does not significantly decrease the coronary vasodilation [90].

**Endogenous** VIP is released in the coronary vessels and heart during parasympathetic (vagal) nerve stimulation and also produces significant coronary artery dilation [78,91,92]. The magnitude of the coronary dilation is directly dependent on the frequency of vagal nerve stimulation [91,92]. In this regard, VIP is most readily released during high frequency vagal nerve stimulation in contrast to the classical neurotransmitter acetylcholine, which is usually released during low frequency nerve stimulation. VIP, released during cardiac vagal nerve stimulation, increases coronary artery blood flow by as much as 62% in dogs in which aortic pressure and heart rate are maintained at a constant level and the muscarinic and β-adrenergic receptors are blocked with atropine and propranolol [92]. Following the termination of vagal stimulation, coronary artery flow returns gradually toward the baseline over 30 min due, most probably, to the slow lymphatic elimination of endogenous VIP [78,91,92]. The increase in coronary artery flow is equivalent to the increase in blood flow that occurs during the intracoronary administration of $9.0 \times 10^{-11}$ mol of VIP or $1.0 \times 10^{-8}$ mol of nitroglycerin [91]. When the VIP antagonist, [4Cl–d-Phe$^6$,Leu$^{-}$]VIP, is directly injected into the coronary artery of these animals, the coronary artery flow does not increase during vagal stimulation [91]. This antagonist is sensitive and selective for VIP and does not inhibit the receptors for glucagon, peptide histidine isoleucine, bombesin, cholecystokinin, calcitonin gene-related peptide, or substance P [91–94].

The effect of endogenous VIP on coronary arterial pressure also has been studied in dogs in which the left coronary artery blood flow is maintained at a constant level and the muscarinic and β-adrenergic receptors are blocked with atropine and propranolol [92]. Vagal stimulation in these animals causes coronary dilation and significantly decreases coronary artery pressure by 17%, even though the major determinants of myocardial oxygen consumption do not change. Moreover, the coronary artery pressure remains 16% below the control value for more than 10 min after the termination of vagal stimulation [92]. However, after the injection of a VIP antagonist directly into the coronary artery, vagal stimulation does not decrease coronary artery pressure [92]. These two investigations suggest that VIP directly dilates coronary arteries by acting on specific VIP receptors in the coronary arteries rather than by acting on muscarinic or β-adrenergic receptors or indirectly producing vasodilation.

The release of VIP in the heart and the VIP concentration in the coronary sinus blood is also increased during coronary artery occlusion and during reperfusion [95,96]. In isolated perfused rat hearts in which coronary perfusion is interrupted for 30 min, the VIP concentration in the coronary effluent increases by 250%, from 1 to $3.5 \times 10^{-12}$ M, during the ensuing 60-min reperfusion period [95]. Moreover, coronary perfusion with VIP immediately prior to the induction of ischemia significantly decreases the myocardial release of creatinine kinase and the formation of hydroxyl radicals, and inhibits calcium overload in cardiac myocytes [95–97]. As a consequence, the postischemic reduction in coronary artery flow is significantly decreased. These studies suggest that VIP promotes local blood flow in the heart during acute myocardial ischemia and may also have a free-radical scavenging effect thereby decreasing possible cardiac...
myocyte calcium overload [95–98]. Inhibition of nitric oxide appears to decrease the cardioprotective properties of VIP [97]. This suggests that VIP may act in the heart, in part, through nitric oxide.

The VIP plasma concentration also increases in patients with acute coronary occlusion. In patients with acute myocardial infarction, the VIP concentration in the plasma may increase by 33–62% within 6 h of the onset of symptoms but then abruptly decreases below the normal concentration after 24 h [99,100]. VIP reaches its lowest plasma concentration 48 h after the onset of symptoms of myocardial infarction and then gradually returns to the normal concentration by day 14 [99,100]. With acute coronary occlusion, VIP is released from neurons in the coronary vessels and myocardium, and may also be released from the splanchnic viscera, and can act as a vasodilator to reduce myocardial ischemia. In this manner, VIP can counteract the vasoconstrictive effects of the sympathetic and renin–angiotensin system [95,96,98]. The abrupt decrease in the VIP plasma concentration 24–48 h after the onset of infarction is due to either depletion of VIP from nerve endings or to the impairment in the neurogenic synthesis and release of VIP in the heart because of depletion of high energy phosphates [100]. In patients who die from acute myocardial infarction, the VIP plasma concentrations do not normalize but rather remain significantly lower than the VIP concentrations of patients who survive [100].

The vasodilator effect of VIP is not limited to the coronary arteries. Endogenous or exogenous VIP also produces significant arterial dilation in other body organs. For example, exogenous VIP significantly increases cerebral arterial blood flow as well as blood flow to the eyes, parotid, thyroid and pancreatic glands [101–103]. In addition, endogenous VIP significantly increases blood flow to the salivary glands and the uterus [104,105].

2.2. Cardiac inotropic effects of VIP

VIP has a primary positive inotropic effect on cardiac muscle that is enhanced by its ability to reduce systemic arterial mean pressure by 10–15%, thereby facilitating ventricular–vascular coupling [84,106,107]. When added to isolated atrial or ventricular muscle in tissue baths, VIP, in doses of 10^{-8}–10^{-5} mol, augments developed isometric force by more than 40% and is equal to or greater than isoproterenol in enhancing ventricular muscle contractile force [108,109]. Exogenous VIP is similar to isoproterenol and forskolin in increasing the rate of change of ventricular pressure per unit time (dP/dt) in research animals in which the cardiac output, arterial pressure, and heart rate are held constant [89,110,111]. In patients, VIP, given intravenously in doses of 4×10^{-10} mol/kg/h, increases the left ventricular shortening fraction by 38% while intracoronary VIP, in doses of 9×10^{-11} mol/min, produces a small but significant increase in left ventricular dP/dt [88,112]. This increase is totally independent of β-adrenergic receptor stimulation [89,108,109]. However, the inotropic response to VIP, but not the coronary vasodilator response, may diminish with increasing VIP dose and/or with time and is probably due to VIP receptor desensitization in the myocardium [113].

Endogenously released VIP increases atrial and predominantly right ventricular contractility. Stimulation of the parasympathetic (vagal) nerves, during muscarinic and β-adrenergic receptor blockade in dogs in which right atrial contractile force is continuously monitored, increases the atrial contractile force by 32% [114]. However atrial contractile force does not increase after the VIP antagonist, [4C1–d-Phe6,Leu11]VIP, is injected into the right atrial muscle by way of the right coronary artery [114]. Vagal nerve stimulation, in a frequency dependent manner, also significantly increases right ventricular contraction and relaxation by 28 and 33%, respectively, but only slightly, but not significantly, increases left ventricular contraction and relaxation in dogs in which the muscarinic and β-adrenergic receptors are blocked and the heart rate controlled [91,92,115]. The right ventricular inotropic and lusitropic response is significantly inhibited by the injection of a VIP antagonist into the right coronary artery [91,115]. These positive right, but not left, ventricular inotropic and lusitropic responses are best explained by the fact that distinct VIP immunoreactive fibers are present in the atria and right ventricle, but the distribution of VIP fibers is not abundant in the left ventricle [80,115,116].

In animal models of heart failure and in patients with cardiomyopathy, the concentration of VIP can decrease in the myocardium by more than 50% [117]. Moreover, the VIP receptor density decreases by as much as 62% and is associated with a similar (62%) decrease in the myocardial contractile response [24,117]. However, the affinity of the remaining receptors for VIP may increase [24]. This increased VIP receptor affinity contrasts with β-adrenergic receptors in heart failure, where the density of β1-adrenergic receptors is decreased and β2-adrenergic receptors are mildly uncoupled [24]. In this regard, changes in cardiovascular VIP receptors or VIP signaling pathways may be important in the pathogenesis of heart failure and hypertension. Interestingly, in patients with congestive heart failure or circulatory shock the plasma concentration of VIP can increase by 400% or more [118,119]. However, this increase in the plasma VIP concentration may be due to gastrointestinal ischemia or significant decreases in the hepatic and/or renal clearance of VIP because not all patients with congestive heart failure or circulatory shock demonstrate an increase in the plasma VIP concentration [118,120].

2.3. VIP effects on heart rate

The discovery that VIP-immunoreactive fibers occur in high density in and around the sinus node and the
atrophicentric node of all mammals strongly suggests that VIP can modulate the electrical responses of the heart and can affect the heart rate [80,121]. The co-release of VIP with acetylcholine in the sinoatrial and atrioventricular nodes may prevent potentially dangerous neurally mediated bradycardia rhythms [66,67]. In this regard, VIP may oppose elevated acetylcholine concentrations in the sinoatrial node that inhibit the pacemaker I cá current and activate muscarinic potassium conductance that might otherwise lead to arrest of spontaneous electrical activity [66].

When VIP is injected directly into the sinoatrial artery of dogs, in which the muscarinic and b-adrenergic receptors are blocked, the heart rate increases over 40 s by as much as 37% [122–124]. The heart rate then returns to the baseline over 5–10 min [122]. The magnitude of the VIP-induced increase in heart rate appears to be inversely related to the extent of vagal cardiac acceleratory activation prior to the injection of VIP [125]. Accelerations in heart rate, similar to those observed in research animals, occur when VIP is given intravenously to patients [112]. In addition, VIP, in a dose dependent manner, can shorten the atrophicentric conduction time by as much as 37%, and can decrease the atrial and ventricular refractory periods by 25 and 10%, respectively, as demonstrated in a dog model [126] but not in a rabbit model [127]. On a molar basis, VIP can have a more potent effect on heart rate than does norepinephrine [122]. Consequently, VIP appears to be one of the most potent positive chronotropic and dromotropic neuropeptides [122]. Moreover, the responsiveness of the cardiac automatic cells to exogenous VIP does not appear to diminish appreciably over time.

The effects of endogenous VIP, released from cardiac vagal nerves, on the heart rate also has been examined [114,115]. In animal investigations, the heart rate increases by 30% over ~50 s during vagal nerve stimulation, in the presence of muscarinic and adrenergic blockade, and then declines by 50% over 5 min after the termination of stimulation [114,115]. The magnitude of the heart rate response is directly dependent on the frequency of vagal nerve stimulation with the maximal response occurring at a stimulation frequency of 20 Hz. In addition, the administration of a VIP antagonist into the sinoatrial node artery, via the right coronary artery, prevents the increase in heart rate [114,115,128]. In an isolated atrial tissue preparation, with atropine and propranolol added to the perfusate, vagal nerve stimulation produces a 22.5% increase in the atrial chronotropic response and an atrial VIP output of 0.05 pmol/min/100 g atrial tissue [129]. Moreover, the atrial VIP output increases as the vagal stimulation frequency is increased and reaches a maximal concentration at a stimulation frequency of 20 Hz [129].

The heart rate increases during vagal stimulation are similar to the heart rate increases produced by exogenously administered VIP. However, the heart rate responses contrast with the heart rate responses to sympathetic stimulation or to exogenous norepinephrine in which the time to peak heart rate and the duration of the tachycardia are much shorter. In addition, the time to peak heart rate and the maximal heart rate response resemble the heart rate responses in subjects with ‘postvagal tachycardia’ and the heart rate responses of research animals with ‘excess tachycardia’ (i.e. the heart rate response in animals after muscarinic blockade minus the heart rate response that occurs after vagotomy) [114,115,123,130]. The transmitter responsible for ‘postvagal tachycardia’ and ‘excess tachycardia’ is most likely VIP rather than the classical neurotransmitters norepinephrine or acetylcholine.

3. Conclusions

The presence of VIP in the vagal centers of the medulla oblongata and the significant concentration of VIP in the branches of the vagal nerves and in the heart and coronary arteries suggest that this peptidergic neurotransmitter plays an important role in the regulation of coronary blood flow, cardiac contraction and relaxation, and heart rate. Moreover, the altered affinity, density, and physiological responsiveness of VIP receptors in heart failure and hypertension suggests that these alterations may have an important pathophysiological function. Additional investigations are necessary to define the precise physiologic role of VIP in the regulation of cardiovascular function.

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