Synergistic Hypotensive Effect of Vasoactive Intestinal Polypeptide and \(\alpha\)-Blockade With Phentolamine

Evidence for Vasoactive Intestinal Peptide \(\alpha\)-Adrenoceptor Coupling in the Cardiovascular System of Newborn Dogs

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Vasoactive intestinal polypeptide (VIP) is a neuropeptide with potent circulatory effects in the adult animal and human. Little is known about its effects or mechanism of action in the immature animal. These series of experiments evaluated the effects and possible mechanism of action of VIP on the developing canine cardiovascular system. In all three series, measurements of mean heart rate and blood pressure were taken in the control state, after parasympathetic denervation with bilateral cervical vagotomies, and after autonomic blockade with propranolol (1 mg/kg) and phentolamine (0.5 mg i.v.). In series 1, we characterized the role of \(\alpha\)-adrenergic receptors in early newborn puppies by investigating the hemodynamic effects of phentolamine alone in five early newborn puppies. In series 2, the hemodynamic effects of intravenous VIP infusion (0.2 \(\mu g/kg/min\)) were recorded and compared in six early newborn puppies and in 10 late newborn puppies. In series 3, the hemodynamic effects of phentolamine in the presence of VIP receptor binding inhibitor were studied. In early newborn puppies, VIP had essentially no effect on heart rate or blood pressure until phentolamine was given; then, blood pressure decreased by 17% \((p<0.005)\). In late newborn puppies, VIP resulted in an increase in heart rate in the control state but not after parasympathetic or sympathetic denervation. In early newborn puppies, phentolamine alone resulted in a 24% decrease \((p<0.005)\) in blood pressure, compared with a 54% decrease \((p<0.005)\) in early newborn puppies preexposed to VIP infusion. VIP receptor binding inhibitor alone had no effect on heart rate or blood pressure but blocked the hypotensive effect of phentolamine even at a dose 10 times higher (5.0 mg phentolamine). It is concluded that 1) VIP has distinctly different effects on the developing canine cardiovascular system from those reported in the adult and 2) VIP and phentolamine have a synergistic hypotensive effect that is abolished by VIP receptor binding inhibitor, suggesting a unique interaction between VIP and \(\alpha\)-adrenoceptors in the newborn cardiovascular system. (Circulation Research 1990;67:986–992)

Vasoactive intestinal polypeptide is a 28 amino acid–residue straight-chain polypeptide that was first identified in porcine lung and later isolated from porcine intestine. However, with the advent of immunohistochemical techniques, VIP has been found to be widely distributed throughout the central and peripheral nervous systems, and its main physiological role is now thought to be that of a neurotransmitter or neuromodulator rather than a simple intestinal hormone. In addition to its wide distribution in the neuroendocrine system, VIP-immunoreactive fibers have been shown to densely innervate specialized tissues of the mammalian cardiovascular system, specifically the major systemic and pulmonary vessels, the atrial and ventricular myocardium, the sinoatrial and atrioventricular nodal conduction tissue, and the coronary vasculature. Moreover, VIP immunoreactivity has been demonstrated within the intrinsic, presumed parasympathetic ganglia of the mammalian heart and is colocalized with classical neurotransmitters. The cardiovascular effects of VIP that have been reported in the adult animal and human include systemic and
coronary arterial vasodilatation and a positive inotropic and chronotropic effect.4,12-15 Although VIP has been reported to exert these effects via a receptor-stimulated increase in adenylyl cyclase activity,16-20 the precise mechanism of action and its probable complex interaction with autonomic regulation of cardiovascular function is unknown.

It is well known that the parasympathetic and sympathetic innervation of the heart and vascular system is not complete at the time of gestation but, rather, follows a developmental course to maturity from several weeks to months after birth, undergoing changes that involve neurotransmitter and receptor acquisition and receptor sensitivity.21-26 There is evidence that neonatal myocardium contains an increased density of α-adrenergic receptors27-29 and that the α-adrenergic effects may also follow a developmental course. Drugge et al29 describe an ontogenetic change in α-adrenergic effects in the rat heart from excitation to inhibition.

Little is known about the developmental course or effects of VIP-immunoreactive fibers and receptors. Since VIP-immunoreactive fibers are closely related anatomically to the autonomic pathways in their cardiovascular distribution,9-11 it is likely that peptidergic receptor acquisition and maturity of innervation may have important developmental significance in the regulation of cardiovascular function in the newborn as well. Furthermore, it is reasonable to assume that the adrenergic and peptidergic effects may interact in their regulatory function.

The purpose of our investigation was twofold: 1) to determine whether VIP has significant hemodynamic effects on the newborn cardiovascular system and 2) to determine whether those effects, if found to be significant, are direct or mediated via adrenergic pathways. The study provides new information about a novel interaction between VIP and α-receptors in the cardiovascular system of neonatal dogs. A preliminary report of some of these results has been presented to the Society for Pediatric Research and the American Academy of Pediatrics, Section of Cardiology.30,31

### Materials and Methods

Three different series of experiments were undertaken. All experiments were carried out on live mongrel puppies of both sexes weighing from 500 g to 3.5 kg. The age distribution of the puppies used in this study is shown in Table 1. The mean ages of the early newborn puppies (ENBPs) and the late newborn puppies (LNBP) were 11.5 and 27 days, respectively, with no overlap in age between the two groups. These ages were chosen to evaluate possible differences in response to the peptide that were indicative of maturational changes in peptidergic innervation, since the autonomic innervation matures over a similar time course.24 Each puppy was anesthetized with 30 mg/kg i.p. sodium pentobarbital, intubated, and mechanically ventilated (model 507 pump, Harvard Apparatus, South Natick, Mass.).

Arterial blood gases were measured to ensure adequate acid/base status. An arterial catheter was placed by a cutdown procedure in the femoral artery and advanced to the proximal descending aorta for the purpose of measuring arterial blood pressure and heart rate by the pulse interval. These parameters were monitored with a pressure transducer (model P23, Gould, Cleveland) and recorded on a preamplifier and pen recorder (model 79, Grass Instrument Co., Quincy, Mass.). A central venous line was inserted into the right internal jugular vein and advanced into the right atrium for the purpose of drug and peptide infusions. The cervical vagi were isolated, dissected free of fascial coverings, and tagged for easy access for vagotomies later in the experiments. A recovery period of 30-45 minutes was allowed after surgical procedures before beginning each of the experimental protocols.

### Series 1: Hemodynamic Effects of Phentolamine in ENBPs

This series of experiments determined the effects of α-adrenergic blockade on the newborn cardiovascular system. In five ENBPs, parasympathetic denervation was performed by severing the cervical vagi, and chemical β-receptor blockade was accomplished by intravenous infusion of propranolol (1 mg/kg Inderal, Ayerst Laboratories, New York). Heart rate and blood pressure were then measured in the control state and after 0.5 mg i.v. phentolamine (Regitine, CIBA Pharmaceutical Co., Summit, N.J.). These doses of propranolol and phentolamine were shown in previous experiments in our laboratory to provide adequate β- and α-blockade when challenged with isoproterenol and phenylephrine.32

### Series 2: Hemodynamic Effects of VIP Infusion on the Developing Canine Cardiovascular System

In these experiments, the effects of VIP infusion were recorded and compared in the ENBP and the LNBP. Sixteen puppies were divided into two groups; there were six ENBPs and 10 LNBP. Heart rate and blood pressure were measured after the following experimental manipulations: 1) in the control state and after 15 minutes of continuous intravenous infusion (microinfusion pump, model 965A, IMED Corp., San Diego) of 0.2 μg/kg/min VIP (lot 011365, Peninsula Laboratories, Inc., Belmont, Calif.), 2) after parasympathetic denervation via bilateral cervical vagotomies in the control state and after 15 minutes continuous VIP infusion, and 3) after total autonomic denervation consisting of vagotomies.
plus autonomic blockade first with 1 mg/kg i.v. propranolol and then with 0.5 mg i.v. phentolamine. A 15-minute interval was allowed between each experimental manipulation to allow sufficient time for washout of the peptide since the reported half-life of VIP is less than 2 minutes.4

In three other ENBPs, 1 mg/kg i.v. atropine was given, followed by a bolus infusion of 30 μg/kg i.v. VIP. Heart rate plus blood pressure were measured to assess the actions of VIP in the presence of postganglionic muscarinic receptor blockade.

Series 3: Hemodynamic Effects of VIP Receptor Binding Inhibitor Alone and α-Blockade With Phenotamine in the Presence of VIP Receptor Binding Inhibitor in ENBPs

In these experiments, VIP receptor blockade with the VIP receptor binding inhibitor (VIP-RBI) L-8-K (lot 014098, Peninsula Laboratories) was produced to determine whether this would alter the α-adrenergic responses and thereby provide evidence for a direct interaction between α-adrenergic and VIP receptors. The VIP-RBI is an 8 amino acid synthetic peptide that has been shown to inhibit VIP binding to receptors in hamster pancreatic tumor cells.33

In this group of five ENBPs, the same protocol was used for parasympathetic and β-adrenergic blockade. Heart rate and blood pressure were measured in the control state, after administration of 30 μg VIP-RBI by bolus infusion, and again after immediate infusion of phenotamine in three increasing doses of 0.5, 2.5, and 5.0 mg.

Statistical Analysis

All data are presented as mean±SD. Results were statistically analyzed by Student’s t test for paired samples and considered significant at p<0.05.

Results

Hemodynamic Effects of Phenotamine in ENBPs

In series 1, the hemodynamic effects of phenotamine, an α-adrenoceptor antagonist, were compared with the control state in the ENBP. Phenotamine (0.5 mg) resulted in a 9% decrease in mean heart rate from 198±18 to 180±33 beats/min, which was not statistically significant. However, phenotamine produced a significant decrease in mean blood pressure from 48±5 to 36±9 mm Hg (n=5; p<0.05), representing a 24% decrease in mean blood pressure (see Figure 1).

Hemodynamic Effects of VIP Infusion on the Developing Canine Cardiovascular System

In this series of experiments, VIP was infused in both ENBPs and LNBPs. In the control state, VIP resulted in a small, but not significant, decrease in mean heart rate from 194±15 to 176±25 beats/min (n=6) in ENBPs; however, in LNBPs, there was a 9% increase in mean heart rate from 188±25 to 206±20 beats/min (p<0.005; n=11) (Figure 2A). This increase in mean heart rate produced by VIP in LNBPs is similar to that previously reported in adult animal studies.12,13

Figure 1. Effects of phenolamine on heart rate and mean arterial pressure in early newborn dogs. The bar graphs represent the mean±SD of results obtained in five early newborn puppies before (control) and after infusion of 0.5 mg i.v. phenotamine. The 24% decrease in mean arterial pressure after phenolamine infusion is statistically significant (p<0.005).

After parasympathetic denervation, VIP had no effect on the mean heart rate in ENBPs (189 beats/min before exposure to VIP and 191 beats/min after 15 minutes of VIP infusion; n=5). However, again there was a 21% increase (p<0.005) in mean heart rate in LNBPs (from 180±27 to 228±34 beats/min; n=9) (Figure 2B). Finally, after total denervation (vagotomies plus sympathetic blockade with propranolol and phenolamine), there was a small decrease (4%) in heart rate in ENBPs (from 152±16 to 146±14 beats/min; n=5) and again an increase (7%) in LNBPs (from 172±21 to 184±42 beats/min; n=9) (Figure 2C); both of these changes did not reach statistical significance.

The mean blood pressure was essentially unchanged by VIP in the control state in both groups of puppies: from 48±4 to 46±7 mm Hg (n=6) in the ENBP and from 76±25 to 75±27 mm Hg (n=11) in the LNPB (Figure 3A). There were similar small, but not significant, decreases in mean arterial pressure after parasympathetic denervation: from 46±6 to 44±3 mm Hg (n=5) in the ENBP and from 75±27 to 74±24 mm Hg (n=9) in the LNPB (Figure 3B). However, there was a significant decrease (17%; p<0.005) in mean blood pressure in the ENBP after total “denervation” (parasympathetic denervation and autonomic blockade), with mean blood pressure falling from 23±4 to 19±4 mm Hg (n=5). In contrast, there was essentially no change in the mean blood pressure of the LNPB (from 70±29 to 68±30 mm Hg; n=9) (Figure 3C).

It is interesting to note that, in the above series of experiments after parasympathetic denervation, the mean blood pressure fell dramatically with 0.5 mg phenolamine in the ENBP (from 48 to 23 mm Hg; n=4); this fall represents a 54% decrease (Figure 4A). However, in the LNPB, 0.5 mg phenolamine had little effect on blood pressure (see Figure 1B). This decrease in mean blood pressure in the ENBP
represents a marked hypotensive effect of phenolamine when compared with the series 1 group of puppies, who were not exposed to intravenous VIP (Figure 4B). Propranolol produced no change in mean blood pressure (from 75 to 70 mm Hg) in either the ENBP or the LNB.

To determine if the effects of VIP were mediated via the cholinergic pathways of the parasympathetic cardiac ganglia, atropine was used to block postsynaptic muscarinic receptors of the atrial myocardium. In the three ENBPs that received atropine before bolus VIP infusion, the mean heart rate dropped from 169±17 to 146±29 beats/min after VIP infusion (Figure 5A), and the mean blood pressure decreased from 36±9 to 23±13 mm Hg (n=3) (Figure 5B). However, the changes in heart rate and blood pressure observed after VIP infusion in the presence of muscarinic receptor blockade did not reach statistical significance.

Hemodynamic Effects of VIP-RBI and Phenolamine in the Presence of VIP-RBI in ENBPs

The rationale for the final series of experiments was based on the observed synergistic hypotensive effect between phenolamine and VIP in the ENBP, suggesting interaction between α-adrenergic and VIP receptors. The following experiments were undertaken to determine if VIP receptor blockade with VIP-RBI would alter the hemodynamic effects of phenolamine in the ENBP.

In this group of five ENBPs, the same protocol was followed for parasympathetic denervation and β-adrenoceptor blockade. Administration of VIP-RBI alone had no significant effect on either mean heart rate or blood pressure. The mean heart rate decreased from 199±19 to 188±24 beats/min, and mean blood pressure decreased from 43±12 to 39±10 mm Hg (n=5) (Figures 6A and 6B). These changes in mean heart rate and blood pressure were not statistically significant. However, when phenolamine was given after VIP-RBI infusion, the hypotensive effect was abolished (Figure 7), even with a phenolamine dose (5 mg) 10 times higher than the dose (0.5 mg) that had previously resulted in a 24% decrease in mean blood pressure.

Discussion

Phentolamine has both α1- and α2-blocking effects on systemic arterial vessels and the myocardium. Although β1-receptors have classically been considered to be the major receptors by which catecholamines exert their effects on cardiac function, new evidence suggests that α-receptor number may be higher in newborn mammalian hearts and may mediate an important positive inotropic and chronotropic

FIGURE 2. Effects of vasoactive intestinal polypeptide (VIP) on heart rate in the early newborn (ENB) and the late newborn (LNB) puppy in the control state (panel A), after parasympathetic denervation with bilateral vagotomies (panel B), and with total autonomic denervation (panel C). The bar graphs represent the mean±SD of results obtained in six ENB and nine LNB puppies. There is a significant increase (p<0.005) in heart rate in the LNB puppy after VIP infusion both in control state and after bilateral vagotomies.

FIGURE 3. Effects of vasoactive intestinal polypeptide (VIP) on mean arterial pressure in the early newborn (ENB) and the late newborn (LNB) puppy in the control state (panel A), after parasympathetic denervation with bilateral vagotomies (panel B), and with total cardiac denervation (panel C). The bar graphs represent the mean±SD of results obtained in five ENB and nine LNB puppies. There was no significant effect of VIP on mean arterial pressure in LNB puppies and only a small decrease in mean blood pressure for ENB puppies with total autonomic denervation.
Similarly, α-receptor blockade with phentolamine has shown to decrease heart rate in newborn puppies via a prolongation of atrial and ventricular refractoriness and to decrease systemic blood pressure, presumably due to a direct effect on myocardial and vascular smooth muscle cells. This response to phentolamine in ENBPs was further supported by our data. Concordantly, these data revealed that VIP produced a small decrease in mean arterial pressure and a negative chronotropic response in newborn puppies. Before the present study, VIP has only been reported to have positive chronotropic activity, although to our knowledge this represents the first study to evaluate the chronotropic response in the newborn in vivo. Clearly, the response to VIP did change with maturation of the animal, suggesting that peptidergic innervation of the cardiovascular system may not be complete at the time of birth.

The present data indicate that, although both phentolamine and VIP appear to have similar hemodynamic effects on newborn circulatory function, there is a marked synergistic effect on systemic blood pressure when they are administered together. This response presumably represents a combined effect on both cardiac and vascular smooth muscle cells, since both VIP and α-receptors are found in high density in those cells. Prior infusion of VIP clearly enhanced the hypotensive effects of phentolamine, and phentolamine also appeared to increase the hypotensive response of VIP. This may suggest an inhibitory relation between α-adrenergic receptors and VIP receptors. This was further supported by the observation that pretreatment with VIP-RBI abolished the hypotensive effect of phentolamine, even when phentolamine was administered at 10 times the dose required to produce significant hypotension in the control ENBP, whereas VIP-RBI alone had no significant effect on either heart rate or blood pressure.

It is proposed that VIP receptors may be coupled to either presynaptic α receptors in an excitatory manner or postsynaptic α receptors in an inhibitory manner to depress the postsynaptic response and produce a hypotensive effect. Thus, VIP may stimulate presynaptic α receptors and inhibit neurotransmitter release, or VIP would inhibit α receptors and reduce neurotransmitter binding. Inhibition of VIP receptor binding by VIP-RBI would block α receptor stimulation and thus facilitate transmitter release, or alternatively, VIP-RBI would block the inhibitory effect on α receptors, thus potentiating neurotransmitter binding and blocking the synergistic hypotensive effect of VIP and phentolamine. The hypothesis that α-adrenergic and peptidergic receptors may be coupled in their cardiovascular responses is also supported by recent data indicating that the release of neuropeptide Y, another cardiac peptide, is enhanced by α-adrenoceptor blockade. Warner and Levy showed that there was a prolonged vagal inhibition by neuropeptide Y in dogs that could be augmented by phentolamine.
FIGURE 6. Bar graphs showing the effect of 30 μg/kg vasoactive intestinal polypeptide receptor binding inhibitor (VIP-RBI) on heart rate (panel A) and mean arterial pressure (panel B) compared with the control state in early newborn puppies (n=5).

VIP has been shown to result in a receptor-mediated increase in cyclic AMP production in multiple experimental models, a mechanism similar to that by which β-receptor activation exerts its cardiovascular effects. A synergistic interaction between VIP and norepinephrine to stimulate cyclic AMP formation and its antagonism by phentolamine, initially described in mouse cerebral cortex,37 has been also recently reported in rat pinealocytes.38 Furthermore, Yuwiler39 described a synergistic action of postsynaptic α-adrenergic receptor stimulation on VIP-induced increases in rat pineal N-acetyltransferase activity. These studies also suggest that there may indeed be VIP and α-adrenergic receptor interactions. α-Adrenoceptor activation, however, acts in a different manner through a calcium-sensitive mechanism triggered by an increase in polyphosphoinositide turnover. Although speculative, the proposed interaction of VIP with α-receptors may be explained by the fact that some hormones and neurotransmitters have been reported to act on more than one receptor type with the ability to interact with both cyclic AMP and calcium-linked pathways. This "conversion phenomenon,"40 a functional coupling between the two types of receptors, may occur with an apparent conversion to one or the other receptor type.22–25,27 It is possible that the conversion is a developmental phenomenon such that VIP mediates its responses by a different mechanism in the newborn than it does in the mature animal.

Regardless of its mechanism of action, it can be concluded from the present data that VIP exerts its cardiovascular effects in the newborn puppy via a complex interaction with α-receptors. Peptidergic innervation to the cardiovascular system may not be complete at birth and appears to follow a developmental course to maturity over the first month of life in the dog, similar to that of the autonomic cardiac innervation.24 Further studies are required to determine the precise relation between peptidergic and adrenergic control of cardiac function and may lead to the development of new therapeutic modalities in the treatment of certain types of cardiovascular diseases.

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