Vasoactive Intestinal Polypeptide Facilitates Atrioventricular Nodal Conduction and Shortens Atrial and Ventricular Refractory Periods in Conscious and Anesthetized Dogs

Dean F. Rigel and David A. Lathrop

Our study was designed to determine the cardiac electrophysiological influence of vasoactive intestinal polypeptide (VIP) in conscious dogs. Dogs (n=8) were chronically instrumented with arterial and venous catheters, cervical vagal cooling coils, and right atrial and right ventricular bipolar epicardial pacing and recording electrodes. After autonomic blockade (10 mg/kg i.v. hexamethonium, 0.11 mg/kg i.v. atropine, and vagal cold blockade), VIP (50 and 100 pmol/kg/min i.v.) or isoproterenol (ISO) (250 and 500 pmol/kg/min i.v.) increased heart rate (maximum increases: VIP, 81.1±4.2 beats/min; ISO, 61.3±8.5 beats/min), decreased the atrial-ventricular interval (during constant atrial pacing) (VIP, −41.9±6.3 msec; ISO, −34.6±7.4 msec), shortened the atrial effective refractory period (VIP, −24.4±2.1 msec; ISO, −30.6±4.4 msec) and ventricular effective refractory period (VIP, −4.2±0.7 msec; ISO, −10.0±2.4 msec), and decreased mean arterial pressure (VIP, −51.9±4.0 mm Hg; ISO, −26.1±2.4 mm Hg). β-Adrenergic blockade with propranolol (1 mg/kg i.v.) eliminated the positive chronotropic and atrioventricular nodal dromotropic responses to bolus doses of ISO (30, 100, 300, and 1,000 pmol/kg i.v.) but did not affect the responses to VIP (10, 30, 100, and 300 pmol/kg i.v.). Comparable blood pressure decreases produced by sodium nitroprusside caused only minimal changes in heart rate, atrial-ventricular conduction times, and atrial and ventricular refractory periods. In three additional anesthetized dogs, after vagotomy and β-adrenergic blockade (1 mg/kg i.v. propranolol), VIP (100 pmol/kg/min i.v.) shortened the atrial-His interval but did not alter intra-atrial, intraventricular, or His-Purkinje conduction. Our findings combined with the demonstration by others of VIP-immunoreactive nerves innervating canine sinus nodal cells, atrioventricular nodal cells, and atrial and ventricular myocardial cells suggest that endogenous VIP may directly alter the electrical properties of the heart. (Circulation Research 1990;67:1323–1333)

Vasoactive intestinal polypeptide (VIP) is a 28-residue peptide that is structurally related to secretin and glucagon. VIP-immunoreactive (VIP-IR) neurons have been detected in hearts of various mammalian species including dog, cat, rat, guinea pig, monkey, and human.1–3 VIP-IR nerves are especially abundant around the sinus node, the atrioventricular node, and atrial myocardium but are less dense in ventricular myocardium.3 The distribution pattern of VIP-IR resembles that of parasympathetic nerves; thus, VIP is probably a cotransmitter with acetylcholine in the heart as it is in other organ systems.3,4

Previously, we described a pronounced positive chronotropic effect of intracoronary VIP in the in situ canine heart.5 VIP was equally effective (heart rate increase of 120 beats/min) and twice as potent (based on ED50 values) as norepinephrine as a positive chronotropic agent. Similarly, we have recently reported6 that VIP augments developed isometric force, rate of force development, and rate of relaxation in isolated canine atrial and ventricular trabecular muscles. VIP was nine and three times more potent than isoproterenol in atrial and ventricular

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muscles, respectively. Both the positive chronotropic and inotropic actions of VIP were unaffected by prior \( \beta \)-adrenergic receptor blockade. Based on the pronounced positive chronotropic and inotropic effects of exogenous VIP and the presence of VIP-IR nerves throughout the canine cardiac conduction system, it is likely that VIP also exhibits important dromotropic effects. Therefore, in the present study we sought to evaluate the actions of exogenous VIP on the electrophysiological characteristics of the intact canine heart. Specifically, the effects of intravenous VIP (bolus injections and infusions) on atrioventricular nodal conduction and on atrial and ventricular refractory periods were compared with those of isoproterenol in autonomically blocked, conscious and anesthetized dogs.

**Materials and Methods**

**Conscious Dogs**

**Animal preparation.** Eight healthy mongrel dogs of either sex (16–22 kg) were instrumented during aseptic surgery. Dogs were anesthetized with acepromazine (1 mg/kg i.m.) followed 20 minutes later by pentobarbital sodium (15 mg/kg i.v.). Surgical and experimental procedures were conducted in accordance with guidelines established by the Institutional Animal Care and Use Committee at the University of Cincinnati.

Polyvinylchloride catheters (0.050-in. i.d. \( \times \) 0.090-in. o.d.) were inserted into an omocervical artery and vein. The neck was opened through a midline ventral incision to allow implantation of vagal cooling coils according to the method of Bishop and colleagues. A length of approximately 10 cm of each vagosympathetic trunk was dissected free from each carotid artery. A layer of tissue was sutured over the carotid arteries to isolate these vessels from the nerves. Cooling coils were constructed from 10-gauge stainless-steel hypodermic tubing as described earlier. Coils were spiraled around each nerve and insulated from the surrounding tissue by a medical-grade silastic sleeve placed around each coil. Polyvinylchloride tubing was attached to each end of each coil to allow circulation of a cold (\( -8^\circ \) to \( -10^\circ \) C) antifreeze solution (95% ethanol) through the coils during subsequent experiments. Thermistors (model YSI 44018, Yellow Springs Instrument Co., Yellow Springs, Ohio) were implanted inside each coil adjacent to the nerve for monitoring approximate vagal nerve temperature. Conduction blockade was considered complete at a nerve temperature of 0–2\( ^\circ C \) and was confirmed by the lack of heart rate change in response to muscarinic receptor blockade (0.11 mg/kg i.v. atropine methylbromide).

After tracheal intubation and initiation of artificial ventilation, the chest was opened at the right fourth intercostal space. The pericardial sac was opened, and a pericardial cradle was created. Three right atrial and three right ventricular bipolar stainless-steel electrodes were sutured to the epicardium. Atrial electrodes were positioned at the right atrial appendage/superior vena caval junction, at the caudal right atrial appendage/right atrial junction, and at the inferior vena caval/right atrial junction. Ventricular electrodes were attached to the lateral right ventricular wall near the right coronary sulcus. The pericardium was closed loosely over the electrodes. Electrode leads were externalized via the second intercostal space. The chest was subsequently evacuated and closed according to standard technique.

Catheters, cooling coil tubes, thermistor leads, and cardiac electrode leads were tunneled subcutaneously and exteriorized in the dorsal cervical region. The dogs were allowed to recover for at least 2 weeks before initiating experiments. During the recovery period, the dogs were acclimated to the laboratory environment and trained to lie quietly on their sides. At least 3 days of recovery were allowed between consecutive experiments.

**Data collection.** Beat-by-beat heart rate was obtained by triggering a biotachometer coupler (Gould Instruments, Cleveland) with a surface lead II electrocardiogram. Systemic arterial pressure was measured with a Gould P231D transducer and the omocervical arterial catheter. Mean arterial pressure was derived by electronic integration of the pulsatile pressure signal. Signals were displayed on a Gould strip-chart recorder (model 2800). Drugs were administered through the omocervical venous cannulas.

Bipolar atrial and ventricular electrograms were amplified (model EA-8 amplifier, Medical Electronics Consulting Associates, Indianapolis) at a bandwidth of 1.2–500 Hz. Electrograms were displayed on the strip-chart recorder and a four-channel oscilloscope (model R5115, Tektronix, Beaverton, Ore.). Atrial-ventricular (AV) conduction time was measured either by hand analysis of the electrograms at a chart speed of 200 mm/sec or with a computer program that detected the upstroke of the atrial and ventricular electrograms. Electrogram signals were digitized at a rate of 1,000 Hz with a micro PDP 11-73 computer (Scientific Micro Systems, Mountain View, Calif.) and an analog-to-digital convertor (Andromeda Systems, Canoga Park, Calif.). Computed AV interval was output to the strip-chart recorder on a beat-by-beat basis by means of a digital-to-analog convertor (Andromeda Systems). Cardiac pacing for determination of AV conduction times at a fixed atrial rate and for measuring atrial and ventricular refractory periods was implemented with a stimulator system (series 850, World Precision Instruments, New Haven, Conn.). Square-wave pulses having a width of 2 msec were delivered by a constant-current source at a magnitude of 1.5 times diastolic threshold current.

Atrial and ventricular refractory periods were determined by means of the extrastimulus method. Stimuli were applied to either an atrial or ventricular electrode at a constant basic drive interval (S1-S2). After every tenth S1 stimulus a premature stimulus (S2) was delivered to the same electrode site. Ini-
tially, the S<sub>1</sub>-S<sub>2</sub> interval was intentionally set too short to allow activation. The S<sub>1</sub>-S<sub>2</sub> was progressively incremented by 1-msec steps. Effective refractory period was defined as the longest S<sub>1</sub>-S<sub>2</sub> interval that failed to capture the atrium or ventricle. Functional refractory period was defined as the shortest obtainable A<sub>1</sub>-A<sub>2</sub> or V<sub>1</sub>-V<sub>2</sub> interval.

**Protocols.**

**BOLUS INJECTIONS.** Dose–response curves were generated with VIP and isoproterenol (ISO) after autonomic blockade to assess the potential direct cardiac effects of these agents. Autonomic blockade was achieved by administering 10 mg/kg hexamethonium (followed by 10 mg/kg/hr i.v.) and 0.11 mg/kg atropine methylbromide (followed by 0.11 mg/kg/hr i.v.) followed by bilateral vagal cold blockade.

Before each injection, control heart rate, mean arterial pressure, and AV conduction times were measured. Conduction times were obtained during atrial pacing at a fixed basic cycle length (210–290 msec) that resulted in a pacing rate approximately 100 beats/min greater than the “intrinsic” heart rate. Bolus injections of VIP (10, 30, 100, and 300 pmol/kg i.v.) and ISO (30, 100, 300, and 1,000 pmol/kg i.v.) were administered after allowing sufficient time between doses for the variables to return to baseline. At the peak heart rate response, atrial pacing was again initiated at the same basic cycle length and conduction times measured. To control for the arterial pressure decrease with both agents, cardiac variables were also measured after decreasing arterial pressure with a bolus injection of sodium nitroprusside (10 μg/kg i.v.). This entire procedure was repeated after β-adrenergic blockade with 1 mg/kg propranolol (followed by 1 mg/kg/hr i.v.).

**INFUSIONS.** In a separate series of experiments, VIP and ISO were also infused to allow measurement of atrial and ventricular refractory periods. In addition, heart rate, mean arterial pressure, and AV intervals (with and without atrial pacing) were obtained. During refractory period and AV conduction time determinations, atrial and ventricular pacing cycle lengths were fixed for each dog at 240–270 msec.

Electrophysiological and hemodynamic variables were measured during the following five periods: 1) at baseline, 2) after autonomic blockade was achieved, as described for the bolus experiments, 3) after infusion of VIP at a low rate (50 pmol/kg/min i.v.), 4) after infusion of VIP at a high rate (100 pmol/kg/min i.v.), and 5) after discontinuation of VIP infusion for a sufficient time (~20–30 minutes) to allow heart rate and arterial pressure to return to control levels. Control and response measurements were repeated after administration of 1 mg/kg propranolol (followed by 1 mg/kg/hr i.v.) and VIP (50 pmol/kg/min i.v.), respectively.

On a separate day, the above protocol was repeated in each dog, substituting a low (250 pmol/kg/min i.v.) and high (500 pmol/kg/min i.v.) infusion rate of ISO for VIP. The order of the VIP and ISO experiments was randomized. In these experiments ISO was not repeated after propranolol. Instead, variables were measured before and after infusion of sodium nitroprusside (10 μg/kg/min i.v.) as a control for the arterial pressure decrease that occurs with VIP and ISO.

**Anesthetized Dogs**

**Animal preparation.** Three additional mongrel dogs (10, 15, and 17 kg) were anesthetized with pentobarbital sodium (30 mg/kg i.v.). Supplemental doses of pentobarbital were administered to maintain a stable plane of anesthesia. Rectal temperature was monitored and maintained within ±0.2°C of baseline by means of a heating pad. Electrodes were attached to the dog to obtain a surface lead I electrocardiogram.

Cannulas were inserted into a femoral vein and artery for administering drugs and peptide and for monitoring arterial pressure, respectively. The cervical vagosympathetic trunks were ligated and sectioned bilaterally. After tracheal intubation and initiation of artificial ventilation with 100% O<sub>2</sub>, the chest was opened at the right fourth intercostal space. The pericardium was opened, and the heart was suspended in a pericardial cradle. Bipolar plunge electrodes were attached to the right atrium near the sinus node and to the lateral right ventricular wall for recording high atrial and ventricular electrograms, respectively. An additional bipolar plunge electrode was attached to the right atrial appendage for pacing the heart. A quadrupolar catheter electrode was inserted into the contralateral femoral vein and advanced to the right atrium for recording His bundle electrograms.

**Data collection.** Cardiac electrogram, His catheter, and electrocardiographic signals were amplified with electrocardiographic/electroencephalographic/His amplifiers (Electronics for Medicine, Pleasantville, N.Y.). Electrograms and His recordings were filtered at 30–500 Hz. Pulsatile arterial pressure was monitored with a Gould P231D transducer and an Electronics for Medicine pressure amplifier. Signals were monitored continuously on an oscilloscope and recorded as needed on paper at a speed of 150 mm/sec (AR-6 Simultrace Recorder, Electronics for Medicine). Atrial pacing was implemented with a programmable stimulator (model 5325, Medtronic Inc., Minneapolis, Minn.).

Spontaneous cardiac cycle length (AA interval) was measured from the atrial electrogram signal. Atrioventricular nodal conduction time was estimated from the AH interval measured from the atrial deflection in the His bundle recording to the His potential. His-Purkinje conduction (HV interval) was derived from the His potential to the ventricular deflection in the His bundle recording. Intra-atrial (A-A<sub>H</sub>) and intraventricular (V<sub>H</sub>-V) conduction times were measured from the atrial electrogram to the atrial deflection in the His recording and from the ventricular depolarization of the His recording to the ventricular electrogram, respectively. Measure-
ments were obtained in triplicate and averaged to derive the corresponding times.

Protocol. Before initiating the protocol, β-adrenergic receptors were blocked with 1 mg/kg propranolol (followed by 1 mg/kg/hr i.v.). The efficacy of the blockade was established by the lack of response to isoproterenol (300 pmol/kg i.v.), which shortened AA and AH intervals before blockade.

Baseline variables (AA, AH, HV, A-AH, and V_H-V intervals) were recorded as control measurements. Atrial pacing was initiated at a rate approximately 100 beats/min greater than the spontaneous heart rate, and baseline variables were again recorded. VIP infusion was initiated and maintained at a constant rate (100 pmol/kg/min i.v.). After 10 minutes of infusion, the variables were again recorded with the heart unpaced and paced. VIP infusion was terminated for 20–30 minutes, and the variables were once again recorded (recovery) as described for the control and VIP infusion measurements.

Data Analysis

Results are presented as mean±SEM. All differences were considered statistically significant at a level of p<0.05.

Conscious dogs. Differences between the bolus dose–response curves before and after propranolol were evaluated with two-way analysis of variance. The two factors consisted of the dose (four levels for each agent) and the status of β-adrenergic receptor blockade (no propranolol versus propranolol).

For the VIP and ISO infusion experiments, data obtained before autonomic blockade and after the low and high doses were compared with the average of the autonomic blockade (i.e., control) and postinfusion recovery data by means of a repeated-measures one-way analysis of variance. When the overall comparison was statistically significant, individual differences were determined by the Bonferroni method of multiple comparisons. Differences between the variables before and after β-blockade were evaluated with paired t test.

Significance of changes in variables before and after decreasing arterial pressure with sodium nitroprusside was determined by paired t test.

Anesthetized dogs. Significance of changes in cardiac intervals was evaluated by comparing the average of the control and recovery values with the values after VIP infusion by paired t test.

Chemicals

Porcine VIP (Bachem, Torrance, Calif.) was dissolved in water and diluted to the appropriate concentration in 0.9% saline. L-Isoproterenol (Isuprel, Winthrop-Breon Laboratories, New York) was used as the commercial preparation diluted in 0.9% saline. Hexamethonium (Sigma Chemical Co., St. Louis), atropine methylbromide (Sigma), propranolol hydrochloride (Sigma), and sodium nitroprusside (Sigma) were dissolved in 0.9% saline.

Results

Conscious Dogs

Bolus injections. To illustrate the time course of responses, we injected VIP and ISO in a dog both while the heart was unpaced (Figure 1, upper tracings) and while the right atrium was continuously paced (Figure 1, lower tracings) at a cycle length of 250 msec (i.e., 240 beats/min). Beat-by-beat heart rate, AV interval, and arterial pressure responses to VIP (100 pmol/kg) and ISO (300 pmol/kg) are shown in the left and right groups of tracings, respectively. The time course (1 minute) of the recordings is indicated in the lower right corner of the figure.

In this example, injection of VIP caused a pronounced heart rate increase from 158 to 217 beats/min, a slight shortening of AV conduction time from 100 to 88 msec (i.e., a facilitation of atrioventricular nodal conduction), and a decrease in arterial pressure from 128 to 40 mm Hg. Each of these responses was transient and followed a similar prolonged recovery time of approximately 8–9 minutes. During atrial pacing, the same dose of VIP produced a pronounced shortening of AV conduction time from 148 to 120 msec and again a hypotension from 97 to 47 mm Hg.

Responses to ISO were similar to those produced by VIP. ISO increased heart rate from 143 to 205 beats/min and decreased arterial pressure from 117 to 35 mm Hg but only slightly decreased AV conduction time (from 98 to 93 msec). As with VIP, the shortening of AV conduction time was more pronounced (from 154 to 111 msec) during atrial pacing at a fixed rate. ISO responses exhibited a more rapid onset and a more rapid return to baseline than the VIP responses (Figure 1).

Figure 2 depicts the composite dose–response data obtained from eight dogs. Both VIP and ISO caused a similar dose-related increase in heart rate (Figure 2A) and a shortening of AV conduction time during atrial pacing (Figure 2B). The highest doses of VIP and ISO increased heart rate by 82.8±3.4 and 83.6±7.1 beats/min and shortened the AV interval by 55.7±8.2 and 55.5±7.1 msec, respectively. Both agents also decreased arterial pressure in a dose-dependent manner. The maximal decreases with VIP, however, were greater than those with ISO (–69.1±6.3 versus –49.2±5.5 mm Hg). Based on the relative shifts of the dose–response curves, VIP is approximately three times more potent than ISO as a positive chronotropic and atrioventricular nodal dromotropic agent.

After β-adrenergic blockade with propranolol, the ISO effects on heart rate and AV interval were virtually eliminated, but the VIP effects were statistically unchanged (Figure 2). Likewise, the arterial pressure response to ISO was virtually abolished, whereas the response to VIP was only slightly attenuated (p<0.05).

Arterial pressure decreases (~60 mm Hg) with sodium nitroprusside, which were comparable with
those with VIP and ISO, caused only slight changes in heart rate and AV conduction time (Table 1). Although these changes were statistically significant in two cases (AV conduction time, before propranolol; heart rate, after propranolol), the magnitude of the changes was still less than the responses to the lowest bolus doses of VIP and ISO.

**Infusions.** Figure 3 displays the composite data from the VIP and ISO infusion protocol conducted on seven conscious dogs. The figure includes heart rate, arterial pressure, AV conduction time (atrium paced and unpaced), and atrial and ventricular effective and functional refractory periods from the VIP experiments (left panel) and from the ISO experiments (right panel) conducted on separate days. The data within each plot are arranged according to the temporal sequence of the protocol.

The far left set of data within each panel (Figure 3) depicts the baseline values before autonomic blockade. Administration of hexamethonium and atropine followed by bilateral vagal cold blockade (autonomic blockade) increased heart rate from approximately 79 to 140 beats/min and prolonged both effective and functional atrial refractory periods by approximately 40 msec but did not significantly alter arterial pressure, ventricular refractory period, or AV conduction time (atrium unpaced). During atrial pacing, the pronounced level of background vagal tone (i.e., before autonomic blockade) combined with the high atrial pacing rate resulted in second-degree atrioventricular nodal block, thus preventing us from measuring baseline AV conduction times.

Both VIP and ISO caused significant dose-dependent changes in each variable (Figure 3). The changes in each variable in response to the highest dose of VIP (100 pmol/kg/min) and ISO (500 pmol/kg/min) are summarized in Table 2. Based on pilot data, the doses of VIP and ISO used in this study were selected to augment heart rate by a comparable amount. There was, indeed, no significant difference between the heart rate increases with VIP (81.1±4.2 beats/min) and those with ISO (61.3±8.5 beats/min) (Table 2). As with the bolus experiments, VIP caused a significantly greater hypotension than did ISO. Likewise, VIP caused a twofold greater shortening of AV conduction time (i.e., −20 versus −10 msec) than did ISO when the atrium was unpaced; however, during atrial pacing, the magnitude of the shortening was greater, but a significant difference between the VIP and ISO responses was no longer detectable. Both agents caused equally pronounced decreases in atrial effective and functional refractory periods (−25 to −30 msec). Ventricular effective and functional refractory periods were slightly but significantly shortened by both VIP and ISO (Figure 3, Table 2). Although the magnitude of these changes was twice as great for ISO (−10 msec) than for VIP (−5 msec), this difference achieved statistical sig-
isoproterenol (ISO) (triangles) bolus) of before (unfilled symbols) is the dose of VIP, these data suggest that VIP is approximately five times more potent than ISO in augmenting heart rate and shortening AV interval and atrial and ventricular refractory periods.

A similar arterial pressure decrease (−41.4±4.6 mm Hg) with sodium nitroprusside infusion did not significantly alter any cardiac variable (Table 1).

In four of the dogs, changes in heart rate, AV conduction time (unpaced and paced), and atrial refractory period (effective and functional) in response to the low dose of VIP were compared before and after propranolol (Figure 4). Only the VIP-induced shortening of atrial effective refractory period was slightly but significantly (p=0.03) attenuated by β-adrenergic blockade (before, −14.5±2.4 msec; after, −12.0±3.0 msec).

**Anesthetized Dogs**

Atrial, ventricular, and His bundle electrogram recordings from a single dog are portrayed in Figure 5. The left groups of tracings were recorded with the heart beating spontaneously (unpaced), and the right tracings were recorded during atrial pacing at a fixed interval (270 msec). Data were obtained during control (upper panels) and during VIP infusion (lower panels). His bundle deflections on the His catheter recordings are labeled H in Figure 5.

During control, the spontaneous cycle length was stable at 557 msec (119 beats/min) due to the previously established autonomic blockade. The corresponding baseline AH interval was 82 msec, as depicted in Figure 5. Pacing the heart at an interval (270 msec) much shorter than the spontaneous cycle length increased the AH interval to 127 msec. Infusion of VIP shortened the AA interval to 340 msec and decreased the AH intervals to 42 and 40 msec with the heart unpaced and paced, respectively. Thus, VIP caused a greater shortening in AH interval when the heart was paced because the initial AH interval was higher during pacing. There were no concomitant changes in $\Delta A_H$, $V_H$, or HV intervals with pacing itself or with the VIP infusion.

**Table 1. Changes in Cardiovascular Parameters by Sodium Nitroprusside Bolus and Infusion in Conscious Dogs**

<table>
<thead>
<tr>
<th>Sodium nitroprusside</th>
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<tbody>
<tr>
<td>Bolus (10 $\mu$g/kg i.v.)</td>
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<tr>
<td>ΔMAP (mm Hg)</td>
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<tr>
<td>Paced</td>
</tr>
<tr>
<td>Before PROP</td>
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<td>After PROP</td>
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</table>

All values are mean±SEM; n=8 dogs for bolus experiments, and n=7 dogs for infusion experiments. MAP, mean arterial pressure; HR, heart rate; AV, atrial-ventricular conduction time; ARP, atrial refractory period; VRP, ventricular refractory period; ERP, effective refractory period; FRP, functional refractory period; PROP, propranolol.

*Significantly different from control at p<0.05 by paired t test.
**TABLE 2. Maximum Changes in Cardiovascular Parameters by Vasoactive Intestinal Polypeptide and Isoproterenol Infusions in Conscious Dogs**

<table>
<thead>
<tr>
<th></th>
<th>VIP (100 pmol/kg/min i.v.)</th>
<th>ISO (500 pmol/kg/min i.v.)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial pressure (mm Hg)</td>
<td>−51.9±4.0</td>
<td>−26.1±2.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>81.1±4.2</td>
<td>61.3±8.5</td>
<td>NS</td>
</tr>
<tr>
<td>AV conduction time (msec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unpaced</td>
<td>−20.6±3.9</td>
<td>−10.3±2.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Paced</td>
<td>−41.9±6.3</td>
<td>−34.6±7.4</td>
<td>NS</td>
</tr>
<tr>
<td>Refractory period (msec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial effective</td>
<td>−24.4±2.1</td>
<td>−30.6±4.4</td>
<td>NS</td>
</tr>
<tr>
<td>Atrial functional</td>
<td>−24.8±2.0</td>
<td>−32.5±4.4</td>
<td>NS</td>
</tr>
<tr>
<td>Ventricular effective</td>
<td>−4.2±0.7</td>
<td>−9.7±2.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ventricular functional</td>
<td>−5.0±1.2</td>
<td>−10.0±2.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM; n=7 conscious dogs. Value of p indicates statistical comparison of vasoactive intestinal polypeptide (VIP) versus isoproterenol (ISO) values by paired t test (NS, not significant; difference is significant at p<0.05). AV, atrial-ventricular.
FIGURE 4. Bar graphs comparing changes in heart rate, atrial-ventricular (A-V) conduction time (unpaced and paced), and atrial effective and functional refractory periods before and after β-adrenergic blockade with 1 mg/kg i.v. propranolol (PROP) in response to the low infusion of vasoactive intestinal polypeptide (50 pmol/kg/min i.v.). Data are from four conscious dogs. *Significant (p<0.05 by paired t test) difference between the responses before and after propranolol.

intervals. Termination of VIP infusion for 20–30 minutes resulted in a return of the AA and AH intervals to approximately the same level as during control.

Pacing the three hearts at an interval much shorter than the spontaneous cardiac cycle length (i.e., at 260, 270, and 280 msec) increased the average baseline AH interval from 76.3±3.5 to 105.0±11.5 msec. During VIP infusion, the AH interval decreased to approximately the same value whether or not the heart was paced. Consequently, the shortening of the AH interval with VIP administration was much greater when the heart rate was maintained constant than when the heart rate was allowed to increase. Again, after terminating VIP, the AH interval returned to the control level. Likewise, during pacing, VIP did not alter any of the remaining three variables.

Changes in the five variables with VIP infusion from the average of the control and recovery values were evaluated for statistical significance (Table 3). VIP significantly shortened the AA interval by 185 msec and decreased AH intervals by 29 and 55 msec with the heart unpaced and paced, respectively. These changes in AH intervals are comparable with the decreases in AV conduction times by the same dose of VIP (100 pmol/kg/min) in our conscious, autonomically blocked dogs (Table 2).

Discussion

Cardiac automaticity and conduction properties are known to be controlled by the parasympathetic and sympathetic branches of the autonomic nervous system. In our conscious dogs, before autonomic blockade, heart rate was low, and second-degree atrioventricular nodal block (during atrial pacing) was manifest. Autonomic blockade increased heart rate, facilitated AV conduction (during pacing), and lengthened atrial refractory period indicating the presence of a dominant parasympathetic control to these regions of the heart under basal conditions.10 Isoproterenol augmented heart rate, shortened AV conduction time, and significantly shortened both atrial and ventricular refractory periods, each of which are well-documented actions of β-adrenergic receptor stimulation.10 Thus, our conscious, chronically instrumented dogs responded in the expected fashion to these autonomic pharmacological challenges, confirming the physiological status of our preparation.

FIGURE 5. Electrograms (His bundle [H], high right atrial [A], and ventricular [V]) from an anesthetized dog after vagotomy and β-adrenergic blockade (1 mg/kg i.v. propranolol). Left tracings were recorded with the heart beating spontaneously (unpaced), and right tracings were recorded during atrial pacing at a fixed interval (270 msec). Data were obtained before (control) and during vasoactive intestinal polypeptide (VIP) infusion (100 pmol/kg/min i.v.). VIP shortens the AA and AH intervals but does not alter intra-atrial (A-AH), intraventricular (V-V), or His-Purkinje (HV) conduction times.
Recent findings indicate that autonomic nerves may also contain neuropeptides that are costored and coreleased along with traditional neurotransmitters. Two such peptides, VIP and neuropeptide Y, have consistently been associated with parasympathetic and sympathetic nerve endings, respectively, that innervate various organ systems. Accordingly, VIP-IR nerves have also been identified in

<table>
<thead>
<tr>
<th>Intervals</th>
<th>Control/recovery average</th>
<th>VIP (100 pmol/kg/min i.v.)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA (msec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unpaced</td>
<td>500.0±26.0</td>
<td>315.0±16.8</td>
<td>−185.0±39.0*</td>
</tr>
<tr>
<td>Paced</td>
<td>270.0±5.7</td>
<td>270.0±5.7</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>AH (msec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unpaced</td>
<td>75.7±3.4</td>
<td>46.3±5.4</td>
<td>−29.3±3.8*</td>
</tr>
<tr>
<td>Paced</td>
<td>104.0±7.6</td>
<td>48.7±5.2</td>
<td>−55.3±12.6*</td>
</tr>
<tr>
<td>HV (msec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unpaced</td>
<td>30.0±0.0</td>
<td>29.3±0.7</td>
<td>−0.7±0.7</td>
</tr>
<tr>
<td>Paced</td>
<td>29.7±0.3</td>
<td>30.0±0.0</td>
<td>0.3±0.3</td>
</tr>
<tr>
<td>A-AH (msec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unpaced</td>
<td>28.7±4.9</td>
<td>25.0±2.9</td>
<td>−3.7±2.0</td>
</tr>
<tr>
<td>Paced</td>
<td>28.7±2.3</td>
<td>30.7±0.7</td>
<td>2.0±3.0</td>
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<tr>
<td>V-VH (msec)</td>
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<td></td>
</tr>
<tr>
<td>Unpaced</td>
<td>35.0±7.1</td>
<td>32.3±5.0</td>
<td>−2.7±2.2</td>
</tr>
<tr>
<td>Paced</td>
<td>35.0±6.5</td>
<td>34.3±4.3</td>
<td>−0.7±2.2</td>
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</tbody>
</table>

Values are mean±SEM; n=3 anesthetized dogs. A, atrial; H, His; V, ventricular; A_AH, atrial deflection on His recording; V_VH, ventricular deflection on His recording.

*Significant difference between response to vasoactive intestinal polypeptide (VIP) and the average of control and recovery at p<0.05 by paired t test.
hearts of various mammals. The most dense supply of VIP-IR fibers has been located in the regions of the sinus node and atrioventricular node. VIP-IR neurons innervating atrial myocardi al cells are also relatively abundant, whereas ventricular tissue receives a sparse supply of VIP-IR nerves. This distribution pattern resembles that of the parasympathetic nerves, suggesting that, as in other organs, cardiac VIP is also colocalized with acetylcholine.

Thus, VIP probably functions as a neurotransmitter in the heart as well as in other organ systems. Although we are unaware of any studies to date that have demonstrated release of endogenous cardiac VIP, electrical stimulation of parasympathetic nerves to other organs does cause a frequency-dependent release of VIP. Overall, these observations provide an anatomic and potential physiological substrate for a role of VIP in modifying cardiac function.

In the present study, we sought to determine whether exogenous VIP was capable of directly (i.e., independently of changes in autonomic tone) altering cardiac electrophysiological properties. In our conscious dogs, the highest doses of VIP evoked changes similar to those produced by ISO. VIP augmented heart rate by 80 beats/min, shortened AV conduction time by approximately 50 msec (during pacing), and decreased atrial and ventricular refractory periods by 25 and 5 msec, respectively. Despite these pronounced responses, an earlier study from our laboratory suggests that these changes are submaximal.

In our previous study, intracoronary injection of both VIP and norepinephrine maximally increased heart rate by 120 beats/min. Thus, the increases in heart rate produced by the highest doses of VIP in the present study were only 67% maximum, suggesting that the maximum changes in AV conduction time and atrial and ventricular refractory periods may also not have been achieved. Because of the pronounced hypotension resulting from the intravenous route of administration of VIP, we were restricted to the doses used.

In our conscious dogs, we measured AV interval as an indicator of atrioventricular nodal conduction. The AV interval changes caused by our doses of VIP are most likely due to facilitation of atrioventricular nodal conduction based on our experiments in anesthetized dogs. In these studies, VIP shortened AH intervals but did not alter intra-atrial, intraventricular, or His-Purkinje conduction. This observation is consistent with previous canine studies that indicate that sympathetic nerve stimulation facilitates atrioventricular nodal conduction but does not appreciably alter atrial or ventricular conduction. Our preparation did not allow us to determine the specific region of the atrioventricular node that was affected by VIP. However, the observation that autonomic stimuli alter AV conduction by influencing higher (i.e., AN and N) regions of the node suggests that VIP may also act at these sites.

We observed a greater shortening of AV and AH intervals by VIP (and ISO) when heart rate was maintained constant by atrial pacing. Presumably, this occurs primarily because the direct positive dromotropic influence of VIP on the atrioventricular node is partly offset by an indirect slowing of atrioventricular nodal conduction. Thus, as VIP increases the atrial rate (i.e., decreases atrial interval), the impulses arrive at the atrioventricular node in closer succession and earlier in the node’s relative refractory period. In addition, the magnitude of the VIP-induced shortening of AV interval may be exaggerated because of the high atrial pacing rate.

In anesthetized dogs, vagal stimulation shortens the atrial refractory period due to a direct cardiac action of acetylcholine. In our conscious dogs, the basal atrial refractory period was short, presumably because of a dominant parasympathetic tone to the atria. Autonomic blockade abolished the vagal actions and thereby prolonged the atrial refractory period by approximately 40 msec. Subsequently, the highest infusion of VIP returned the atrial refractory period nearly to its basal level. In the canine ventricular conduction system, vagal stimulation or acetylcholine application indirectly prolongs refractory period by antagonizing a concurrent adrenergic shortening. Results from our study indicate that VIP, like ISO, also shortens the ventricular refractory period. Thus, the presumed cotransmitters, acetylcholine and VIP, which exhibit opposing effects on sinus nodal automaticity, atrioventricular nodal conduction, and ventricular refractoriness, act in a parallel fashion on atrial recovery.

The observed cardiac changes produced by VIP were apparently not due to direct or reflex-mediated (indirect) interactions with β-adrenergic or muscarinic and nicotinic cholinergic receptors. Propranolol, in a dose that abolished the positive chronotropic and dromotropic effects of ISO, did not alter the effects of VIP. Similarly, the positive inotropic actions of VIP in isolated atrial and ventricular trabeculae muscles were unaffected by β-adrenergic blockade. Cholinergic withdrawal may also be excluded as a factor in the actions of VIP because the combination of atropine, hexamethonium, and vagal cold blockade eliminated the basal vagal cardioinhibition and prevented additional reflex stimulation by the nitroprusside hypotension. In addition, the inefficacy of the nitroprusside hypotension indicates that the electrophysiological changes produced by VIP may not be ascribed to a direct cardiac effect of the pronounced VIP vasodilation/hypotension.

Instead, these VIP-induced changes were most likely due to the direct interaction of VIP with VIP receptors. Membrane receptors to VIP have been demonstrated in atrial and ventricular tissue from a variety of mammalian species, including the dog and human. A functional role for these receptors is suggested by their altered affinity, density, and pharmacological responsiveness in pathological states such as heart failure and hypertension. Moreover, these receptors are functionally coupled to adenylate cyclase, an observation that could explain the qualita-
tively similar actions of VIP and ISO. In the dog, VIP is equal in potency with ISO in stimulating adenylate cyclase activity, whereas VIP is three to nine times more potent than norepinephrine or isoproterenol as a positive chronotrophic, dromotropic, and inotropic agent (present study).

It is well established that the autonomic nervous system plays an important role in predisposing the heart to or protecting the heart from potentially life-threatening arrhythmias. Results of the current study demonstrate that exogenous VIP is capable of inducing electrophysiological changes in the heart of a magnitude comparable with those produced by the classic neurotransmitters. This observation combined with the ubiquitous cardiac distribution of neuronal VIP suggests that endogenous VIP may also modulate the electrical characteristics of the heart and, therefore, influence the development of cardiac arrhythmias.

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References

Key words • vasoactive intestinal polypeptide • isoproterenol • heart rate • atrioventricular nodal conduction • refractory period • arterial pressure • autonomic nervous system
Vasoactive intestinal polypeptide facilitates atrioventricular nodal conduction and shortens atrial and ventricular refractory periods in conscious and anesthetized dogs.

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