Vasoactive intestinal peptide has a direct positive inotropic effect on isolated human myocardial trabeculae

Ole SAETRUM OPGAARD*†, Mikael KNUTSSON†, René de VRIES*, Beril TOM*, Pramod R. SAXENA* and Lars EDVINSSON†
*Department of Pharmacology, Erasmus University, 3000 DR Rotterdam, The Netherlands, and †Department of Medicine, University Hospital, 22185 Lund, Sweden

ABSTRACT
The aim of the present study was to assess the inotropic effects of vasoactive intestinal peptide (VIP) on isolated myocardial trabeculae from the right atrium and the left ventricle of human hearts. Furthermore, using reverse transcriptase–PCR, we wanted to determine the presence of mRNAs encoding the three cloned human VIP receptors, VPAC1, VPAC2 and PAC1. The trabeculae were paced at 1.0 Hz in tissue baths, and changes in isometric contractile force upon exposure to agonist were studied. VIP had a potent positive inotropic effect in some of the atrial and ventricular trabeculae tested. This effect was almost completely blocked by the VIP-receptor antagonist VIP-(6–28). mRNAs encoding the human VPAC1, VPAC2 and PAC1 receptors were detected in human myocardial trabeculae from both the right atrium and the left ventricle. In conclusion, VIP has a direct positive inotropic effect in both the atria and the ventricles of the human heart. The presence of mRNAs for the VPAC1, VPAC2 and PAC1 receptors suggest that VIP may mediate its effect via these receptors.

INTRODUCTION
Vasoactive intestinal peptide (VIP) is present in post-ganglionic parasympathetic nerves that innervate mammalian coronary vessels, endocardium and myocardium [1,2], and can be released from the heart by vagal stimulation [3]. Cardiac vagal nerve stimulation in dogs during muscarinic and β-adrenergic receptor blockade, caused increased contractile force in the right atrium [4] and the right ventricle [5,6], whereas no significant changes were seen in the contractility of the left ventricle [5–7]. It was also shown that intracoronary infusion of the VIP antagonist [4Cl-d-Phe6,Leu17]VIP could significantly reduce the vagal-induced increases in right atrial/right ventricular contractility, suggesting that VIP was the mediator of this positive inotropic effect in the right atrium and ventricle [4,6,8].

Intravenous and intra-arterial infusion of VIP into healthy humans has been shown to cause vasodilatation combined with increased heart rate and stroke volume, but it seemed unclear to what extent the increased cardiac contractility was secondary to decreased vascular re-

Key words: inotropic agents, PAC1 receptor, vasoactive intestinal peptide, VPAC1 receptor, VPAC2 receptor.
Abbreviations: Emax, maximum effect obtained with an agonist; PACAP, pituitary adenylate cyclase-activating polypeptide; pEC50, negative logarithm of the EC50 value; RT-PCR, reverse transcriptase–PCR; VIP, vasoactive intestinal peptide.
Correspondence: Dr Ole Saetrum Opgaard, Department of Pharmacology, College of Medicine, 360 Med Surge II, University of California, Irvine, CA 92697-4625, U.S.A. (e-mail osaetrum@uci.edu).
sistance [9–11]. In one of these studies, echocardiograms recorded during the infusion demonstrated increased left ventricular contractility, as defined by the relationship between end-systolic wall stress and shortening fraction, suggesting that VIP also has a direct positive inotropic effect in the left ventricle [9].

Direct positive inotropic effects of VIP have been demonstrated in isolated cardiac tissue from laboratory animals such as the rat [12], dog [13–15] and monkey [16]. VIP also had a positive inotropic effect in the isolated, artificially driven auricle of the human right atrium [17] and in isolated trabeculae from the human right ventricle [18], whereas the direct effect of VIP on human left ventricular tissue is still unclear.

Three human VIP receptors have been cloned: the VPAC₁ receptor [19], the VPAC₂ receptor [20] and the PAC₁ receptor [21]. The VPAC₁ and VPAC₂ receptors have an almost equally high affinity for VIP and for pituitary adenylate cyclase-activating polypeptide (PACAP), which is another member of the VIP/glucagon peptide family, whereas the PAC₁ receptor has a 100–1000 times lower affinity for VIP than for PACAP [22].

The aim of the present study was to assess the direct inotropic effects of VIP on isolated electrically stimulated trabeculae from the right atrium and the left ventricle of human hearts. VIP was also tested in the presence of the potent VIP receptor antagonist VIP-(6–28) [23]. Furthermore, we wanted to determine the presence of mRNAs for the three cloned human VIP receptors VPAC₁, VPAC₂ and PAC₁ in myocardial trabeculae from atria and ventricles of the human heart.

**METHODS**

The investigation conformed with the principles outlined in the Declaration of Helsinki of the World Medical Association. The collection of human tissues was in accordance with institutional guidelines and was approved by the local ethics committee.

**Functional studies**

**General preparations**

Myocardial trabeculae were excised from the inner surface of the right atrium and the left ventricle of human hearts. The Rotterdam Heart Valve Bank (Bio Implant Services Foundation/Eurotransplant Foundation) kindly provided the hearts after removal of the aortic and pulmonary valves for homograft valve implantation. The hearts came from six females and three males (age 17–63 years; mean ± S.E.M. 49 ± 5 years). All donors were previously healthy individuals who had died from cerebrovascular accidents or head trauma. The hearts were initially stored in a chilled, sterile organ protection solution (UW, Eurocollins; or HTK-Bretttschneider) [24] and, prior to experiments, were placed in chilled Krebs buffer of the following composition (mM): NaCl 118, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2 and glucose 8.3. Only trabeculae that were free from the wall of the heart and which had a diameter of < 1 mm were used. Care was taken not to damage the endothelial surface of the tissue. The trabeculae were mounted in organ baths (15 ml) containing the Krebs buffer, which was kept at 37 °C and gassed continuously with a mixture of 95% O₂/5% CO₂ giving a pH of approx. 7.4. The ends of the trabeculae were tied with silk sutures and connected to a Harvard transducer for measurement of isometric tension. The trabeculae were paced at 1.0 Hz using field stimulation (5 ms; voltage 20% above threshold for initiation of the contractile response), through electrodes placed in the organ baths. Resting tension was set to approx. 750 mg for atrial trabeculae and 1950 mg for ventricular trabeculae [25]. During continuous pacing, the tissues were allowed to stabilize for approx. 90 min before the baseline contractile amplitude was measured.

**Concentration–response curves**

Concentration–response curves for noradrenaline were obtained in some of the trabeculae, showing that a concentration of 10 μM noradrenaline gave a near-maximum response. This concentration of noradrenaline was used to test the responsiveness of all trabeculae and for comparison with responses to VIP. Trabeculae that showed an increase in contractile force of less than 25 mg upon exposure to 10 μM noradrenaline were excluded from the study.

After several wash-outs with normal Krebs buffer and stabilization at baseline contractile force, cumulative concentrations of VIP were added and changes in contractile force were measured. At the end of the experiments, the reactivity of the trabeculae was again tested by exposure to noradrenaline (10 μM).

**Analysis of data**

The maximum effect obtained with an agonist (Eₘₐₓ) and the negative logarithm of the EC₅₀ value (pEC₅₀) were derived from concentration–response curves on each trabecula. Values are given as means ± S.E.M. Student’s t test was used to determine statistical significance with respect to differences in Eₘₐₓ and pEC₅₀ values. Statistical significance was assumed at P < 0.05.

**Drugs**

Noradrenaline was obtained from Sigma (St. Louis, MO, U.S.A.), and VIP (human, porcine) and VIP-(6–28) (porcine) were from Bachem (Bubendorf, Switzerland). The drugs were dissolved in distilled water.
An total RNA were determined by spectrophotometry using as only one atrial and one ventricular trabecula showed a positive inotropic response to VIP after incubation with VIP-(6–28).

There were no significant differences in pEC50 values between atrial and ventricular trabeculae. Incubation with the VIP-receptor antagonist noradrenaline (NA; 10 μM) almost completely abolished positive inotropic responses to VIP in both atrial and ventricular trabeculae. Statistical analysis was not performed, as only one atrial and one ventricular trabecula showed a positive inotropic response to VIP after incubation with VIP-(6–28).

### Table 1 Positive inotropic effects of VIP on isolated human trabeculae from the right atrium and the left ventricle, and effects of preincubation with the receptor antagonist VIP-(6–28) (1 μM)

All agonists were tested at baseline contractile force. n1 is the number of trabeculae tested, and n2 is the number of trabeculae that responded to VIP. Baseline contractile amplitude was measured before exposure to agonist, and is given in units of mg. Values are means ± S.E.M. The increase in contractile amplitude after exposure to noradrenaline (NA; 10 μM) was measured as a percentage of baseline contractile amplitude. Emax values represent the maximum increase in contractile amplitude induced by VIP, measured as a percentage of baseline contractile amplitude, and were calculated from all trabeculae tested (n1), and from only those trabeculae responding to VIP (n2) (all trabeculae responded to noradrenaline). *Significant difference (P < 0.05) in mean Emax value for those trabeculae responding to VIP in atrial compared with ventricular trabeculae. There were no significant differences in pEC50 values between atrial and ventricular trabeculae. Incubation with the VIP-receptor antagonist VIP-(6–28) (1 μM) almost completely abolished positive inotropic responses to VIP in both atrial and ventricular trabeculae. Statistical analysis was not performed, as only one atrial and one ventricular trabecula showed a positive inotropic response to VIP after incubation with VIP-(6–28).

<table>
<thead>
<tr>
<th>Agonist</th>
<th>n1</th>
<th>n2</th>
<th>Baseline amplitude (mg)</th>
<th>Increase due to NA (% of baseline)</th>
<th>Emax (% of baseline)</th>
<th>pEC50</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIP</td>
<td>7</td>
<td>4</td>
<td>50 ± 21</td>
<td>522 ± 111</td>
<td>25 ± 9</td>
<td>7.95 ± 0.25</td>
</tr>
<tr>
<td>VIP-(6–28) + VIP</td>
<td>6</td>
<td>1</td>
<td>65 ± 24</td>
<td>440 ± 82</td>
<td>3 ± 3</td>
<td>7.34</td>
</tr>
<tr>
<td><strong>Ventricles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIP</td>
<td>9</td>
<td>5</td>
<td>159 ± 44</td>
<td>321 ± 65</td>
<td>15 ± 5</td>
<td>7.74 ± 0.10</td>
</tr>
<tr>
<td>VIP-(6–28) + VIP</td>
<td>7</td>
<td>1</td>
<td>197 ± 73</td>
<td>262 ± 68</td>
<td>2 ± 2</td>
<td>7.13</td>
</tr>
</tbody>
</table>

### Molecular biology studies

**Tissue**

Tissue samples were obtained from an explanted heart from a 49-year-old man suffering from dilated cardiomyopathy and undergoing heart transplantation. After collection, the samples were immediately snap-frozen and stored at −86 °C until use.

**RNA extraction**

TRIzol reagent (Gibco BRL, Life Technologies) was used for extraction of total cellular RNA. The frozen samples were treated with 1 ml of TRIzol (100 mg of tissue/ml of TRIzol) and homogenized using the microprobe of a power homogenizer (Polytron Kinematica, Model PT 1200; Polytron, Labora, Sweden). Following the addition of chloroform, the homogenates were centrifuged at 12000 g for 30 min at 4 °C. The aqueous phase containing RNA was transferred to a fresh tube, and RNA was precipitated using propan-2-ol. After incubation at room temperature for 15 min, the samples were centrifuged at 14 000 g for 30 min at 4 °C. The RNA pellet was finally washed with 75% (v/v) ethanol, air-dried, dissolved in diethyl pyrocarbonate-treated water and stored at −20 °C until use. The purity and amount of total RNA were determined by spectrophotometry using a microcuvette (Genquant; Pharmacia, Uppsala, Sweden). An A260/A280 ratio of > 1.6 was considered to indicate purity.

### Reverse transcriptase–PCR (RT-PCR)

Specific oligonucleotide primers for the human VPAC1, VPAC2 and PAC1 receptors were designed from the published human sequences. Primers, obtained from TAG Copenhagen A/S (Copenhagen, Denmark), were as follows: VPAC1 (269 bp): forward, 5′-TCC GTG GTG TGC TGG GTG AAT A-3′; reverse, 5′-GTG TTG GTG AAG TAG GTG ATG G-3′; VPAC2 (444 bp): forward, 5′-TAC AGC AAA GCA GGA AAC ATA A-3′; reverse, 5′-CAG GAG GGT GTG GAG GTA G-3′; PAC1 (764 bp): forward, 5′-AAC CCT TCC CTC ATT ACT TT-3′; reverse, 5′-CCT TTT GCT GAC ATT CTC TG-3′. The primers were designed to span one or more introns in order to distinguish signals from genomic DNA contamination.

Reverse transcription of total RNA and subsequent amplification was performed with GeneAmp RNA PCR kit reagents (Perkin-Elmer, Foster City, CA, U.S.A.) and a PCR DNA thermal cycler (Perkin-Elmer). The cDNA was obtained by retrotranscription of 1 μg of total RNA in a 20 μl reaction volume using random hexamers as primer. The samples were incubated at 25 °C for 10 min, at 42 °C for 25 min, heated to 99 °C for 5 min, and then chilled to 4 °C for 5 min. A 9 μl aliquot of the resultant cDNA was amplified in a final volume of 50 μl using the following amplification profile: 5 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 1 min at the annealing temperature [55 °C (PAC1), 56 °C (VPAC2) or 57 °C (VPAC3)] and 30 s at 72 °C. After the final cycle, the
temperature was maintained at 72 °C for 7 min to allow final extension.

Analysis of PCR products
A 10 μl aliquot from each PCR product was separated on 1.5% (w/v) agarose gel in 0.5 × TBE (1 × TBE = 45 mM Tris/borate and 1 mM EDTA), stained with ethidium bromide (1 μg/ml) and photographed on a UV-box using a videographic printer (Sony). A 100 bp DNA ladder (Promega) was run in the outside lane to confirm the molecular size of the amplification products. The identity of the PCR products was verified by DNA sequencing, which showed no dissimilarities from the published sequences.

RESULTS
Functional studies
VIP, tested at cumulative concentrations up to 300 nM, increased contractile force in four out of seven atrial trabeculae tested and in five out of nine ventricular trabeculae tested. Table 1 shows mean pEC_{50} values and E_{max} values calculated from responding trabeculae only, as well as values obtained when also including those trabeculae not responding to VIP (all trabecula re-

![Figure 1](image1.png)

**Figure 1** Positive inotropic effects of VIP alone and after preincubation with VIP-(6–28)
Concentration–response curves for VIP alone (seven atrial and nine ventricular trabeculae) and after preincubation with the VIP-receptor antagonist VIP-(6–28) (1 μM) (six atrial and seven ventricular trabeculae), tested on human trabeculae from right atria (A) and left ventricles (B). Each point shows the mean increase in contractile amplitude measured as percentage of baseline contractile amplitude for each individual trabecula. The S.E.M.s are shown as vertical bars. Both those trabecula responding and those not responding to VIP are included in the calculation (for ratios of responding to non-responding trabeculae, see Table 1).

![Figure 2](image2.png)

**Figure 2** Gel electrophoresis of RT-PCR products corresponding to mRNAs encoding the VIP/PACAP receptors VPAC1, VPAC2, and PAC1 in the left ventricle and the right atrium of the human heart
Lanes 1 and 8, 100 bp ladder (the band with the greatest intensity indicates 500 bp); lane 2, VPAC1 (269 bp) in left ventricle; lane 3, VPAC2 (444 bp) in left ventricle; lane 4, PAC1 (764 bp) in left ventricle; lane 5, VPAC1 in right atrium; lane 6, VPAC2 in right atrium; lane 7, PAC1 in right atrium.
VIP-receptor antagonist VIP-(6–28) (1 μM) almost completely abolished positive inotropic responses to VIP in both atrial and ventricular trabeculae (Table 1 and Figure 1). Statistical analysis was not performed, as only one atrial and one ventricular trabecula showed a positive inotropic response to VIP after incubation with VIP-(6–28).

**Molecular biology studies**

Gel electrophoresis of the RT-PCR products revealed bands of the expected sizes, corresponding to mRNAs encoding VPAC$_1$ (269 bp), VPAC$_2$ (444 bp) and PAC$_1$ (764 bp), in both the right atrium and the left ventricle of the human heart (Figure 2).

**DISCUSSION**

In the present study, we demonstrate a direct positive inotropic effect of VIP on isolated trabeculae from the right atrium and the left ventricle of the human heart. While previous studies have shown a positive inotropic effect of VIP in the electrically driven auricle of the human right atrium [17], as well as in isolated trabeculae from the human right ventricle [18], this is to our knowledge the first study to demonstrate a direct positive inotropic effect of VIP in the human left ventricle in vitro. In laboratory animals, using various experimental settings, a direct positive inotropic effect of VIP on myocardial tissue from different parts of the heart, including the left ventricle, has been demonstrated, and this effect seems to be mediated through increased production of cAMP [8,12–16]. The role of VIP as a potential positive inotropic agent in the left ventricle has been questioned, however, since vagal stimulation of dogs, probably via endogenously released VIP, caused increased contractility of the right atrium and the right ventricle, but not the left ventricle [4–7]. These differences might be explained by the sparse innervation of VIP-immunoreactive nerve fibres in the left ventricle compared with the atria and the right ventricle of canine hearts [8,26]. Nevertheless, it was also shown that intracoronary infusion of VIP in dogs caused coronary vasodilatation without any significant changes in left ventricular contractility [7]. In patients with chest pain, however, intracoronary infusion of VIP caused coronary vasodilatation, with a small but still significant increase in the rate of change of left ventricular pressure per unit time (dP/dt), as well as in peak left ventricular pressure; these changes occurred concomitant with a small but significant decrease in mean aortic pressure [27].

The fact that not all atrial and ventricular trabeculae responded to VIP in our study, although noradrenaline had a positive inotropic effect in all cases, may be explained by differences in the density or functional state of specific receptors. Although the heart donors were supposedly healthy, there may still have been differences between them. Binding studies with VIP have shown increased receptor affinity but decreased receptor density in failing human ventricular myocardium compared with the non-failing heart; the maximal contractile response of right ventricular trabeculae from failing right ventricles was decreased by 61%, and the dose–response curve to VIP was left-shifted by approx. 3-fold [18]. Furthermore, patients with heart failure and coronary disease had lower levels of VIP in the left ventricular myocardium compared with controls [28]. Another possibility is desensitization, and experiments in dog have demonstrated a desensitization of myocardial VIP receptors after 90 min of VIP infusion [14]. The possibility thus cannot be excluded that even shorter periods of VIP exposure could cause desensitization. However, this is not a very likely explanation for why some trabeculae responded to VIP in our experiments and others did not, as the experimental setting was kept constant and VIP was administered only once.

The positive inotropic effect of VIP was almost completely blocked by the VIP receptor antagonist VIP-(6–28), suggesting that this is an effect mediated via VIP receptors. The presence of VIP receptors is supported further by the detection in both the right atrium and the left ventricle of mRNAs encoding the three cloned VIP receptors, i.e. VPAC$_1$, VPAC$_2$ and PAC$_1$. The VIP/PACAP receptors are distributed widely throughout the mammalian body [22], and have been reported to be present also in the human heart [20,29]. To our knowledge, however, no studies have been performed previously that investigated the presence of VIP/PACAP receptors in the myocardium from the right atrium and the left ventricle of the human heart. A positive inotropic effect mediated via these receptors can be explained by the fact that the VPAC$_1$ and VPAC$_2$ receptors are G-protein–coupled receptors that stimulate adenylate cyclase, leading to the accumulation of cAMP and activation of protein kinase A, whereas the PAC$_1$ receptor is coupled to several transduction systems, among them stimulation of the phospholipase C pathway [22,30]. It is important to bear in mind, however, that our molecular experiments were performed with tissue from a subject with dilated cardiomyopathy, whereas the functional experiments were performed with tissue from healthy subjects, and that heart failure seems to decrease the density of VIP receptors in the human heart [18]. The expression of subtypes of VIP receptors might therefore be differently regulated in the failing myocardium compared with the normal myocardium.

In our present study, the $E_{\text{max}}$ values of those trabeculae responding to VIP were significantly ($P < 0.05$) higher in atrial than in ventricular trabeculae, when measured as percentage of baseline contractile amplitude. This suggests a more important role for VIP as a regulator of contractile force in the right atrium.
compared with the left ventricle. The conclusion that VIP has a more important function in the right atrium than in the left ventricle is also supported by previous studies on mammalian cardiac tissue, showing more dense innervation with VIP-immunoreactive nerve fibres in the atria than in the left ventricle [1,8,26]. We also demonstrated previously the induction of greater contractile force by noradrenaline, endothelin and calcitonin gene-related peptide in human cardiac trabeculae from the right atrium compared with those from the left ventricle [31,32]. Given that atrial contraction is particularly important in order to maintain cardiac output and exercise performance under critical conditions, increased responsiveness of the atria to positive inotropic agents may serve a physiological purpose under such conditions [33]. From an interesting clinical point of view, it has been proposed that the increased cardiac output seen during treatment with angiotensin-converting enzyme (ACE) inhibitors may be due in part to increased cardiac levels of VIP [34].

In conclusion, VIP has a potent direct positive inotropic effect in isolated trabeculae from both the right atrium and the left ventricle of the human heart. The inhibition of the positive inotropic effect by the VIP-receptor antagonist VIP-(6–28) and the presence of mRNAs for the three cloned VIP receptors VPAC₁, VPAC₂ and PAC support the involvement of VIP receptors in mediating these positive inotropic responses.

ACKNOWLEDGMENT

This work was supported in part by a grant from the Swedish Medical Research Council (no. 05958).

REFERENCES

Positive inotropic effect of vasoactive intestinal peptide


Received 7 March 2001/4 June 2001; accepted 13 July 2001