The Effects of Apelin on Myocardial Function after Resuscitation of Hemorrhagic Shock in Rats

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ABSTRACT

Apelin is a novel peptide that has recently been established as the only known ligand for the APJ receptor. Apelin has an important regulatory role in cardiovascular homeostasis. Despite recent advances in the understanding of the cardiovascular effects of the apelin-APJ system, the myocardial protective effects of treating with apelin before resuscitation following hemorrhagic shock has not been investigated. The present study investigated the myocardial protective effects of apelin on preventing myocardial contractile dysfunction after hemorrhagic shock. Methods: Male Sprague Dawley rats (300-350 gm) were assigned to 3 experimental groups (n= 6 per group): Normotensive rats (N); Hemorrhagic shock rats (HS); Hemorrhagic shock rats treated with apelin-13 (HS-AP). Rats were hemorrhaged over 60 min to reach a mean arterial blood pressure of 40 mmHg. Rats were treated with 1 ml of 10 nm /L apelin-13 intra-arterially after 60 min hemorrhagic shock. Resuscitation was performed in vivo by the reinfusion of the shed blood for 30 min to restore normo-tension. Left ventricular contractile function was measured in the isolated hearts following hemorrhage and in vivo resuscitation using the Langendorff apparatus. Results. Hemorrhagic shock rats treated with AP exhibited a significant increase in left ventricular generated pressure LVGP (111.20 ± 9.19 mmHg) and + dP/dtmax (589.6 ± 110.68 mmHg/sec) compared with the untreated group. Conclusion.Treatment with apelin before resuscitation improved myocardial contractile function in a hemorrhagic shock model in rats.

تأثير مادة الأبلين على وظائف القلب بعد الإنعاش من الصدمة النزيفية في الجرذان

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المُستلخص

تلعب مادة الأبلين دوراً مهماً في تنظيم توازن الجهاز الدوري القلبي. بالرغم من التقدم الحديث في فهم مادة الأبلين ، لم تستكشف بعد مدى الحماية للمعالجة بالأبلين قبل الإنعاش من الصدمة النزيفية. الهدف من الدراسة الحالية هو دراسة دور مادة الأبلين في حماية عضلة القلب ضد الخلل الوظيفي التقلصي بعد الصدمة النزيفية. تم استخدام فئران سبراج دولي (300-350 جم) والتي تم توزيعها على 3 مجموعات تجارب (عدد 6 في كل مجموعة): مجموعة ضغط الدم الطبيعي، و مجموعة الصدمة النزيفية، و مجموعة الصدمة النزيفية المعالجة بمادة الأبلين. تم نزف الفئران بسحب الدم على مدى 60 دقيقة للوصول إلى ضغط دم 40 مم زئبق. تم علاج الفئران باستخدام 1 ملل من مادة الأبلين (10 نانوم/ل) وذلك بحقنها داخل الشريان بعد 60 دقيقة من الصدمة النزيفية. تم إنعاش الفئران بعد ذلك وذلك بإعادة حقن الدم المسجوب على مدى 30 دقيقة لاستعاد الضغط الطبيعي. تم قياس وظائف البطين اليسر التقلصية في القلب بعد عزله من الجسم بعد الصدمة النزيفية والإنعاش، وذلك باستخدام جهاز اللانجندورف. من نتائج التجربة أن الفئران المعالجة بمادة الأبلين بعد الصدمة النزيفية قد أظهرت زيادة هامة في وظائف البطين الأيسر التقلصية بالمقارنة بالمجموعة النزيفية الغير معالجة، خلاصة البحث هي أن العلاج باستخدام مادة الأبلين قبل الإنعاش من الصدمة النزيفية حسنت وظائف القلب التقلصية بعد الصدمة النزيفية في نموذج فئران التجارب.

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الكلمات الدالة

الأبلين، وظائف القلب، الصدمة النزيفية، فئران سبراج، جهاز اللانجندورف

Introduction

Hemorrhagic shock and resuscitation is well known to result in myocardial contractile dysfunction and failure (Kline, *et, al.,* 1999). Apelin is a novel endogenous ligand for the G-protein-coupled receptor APJ. G protein-coupled receptors (GPCRs) represent the largest group of transmembrane proteins responsible for transduction of diverse array of extracellular signals (Szokodi *, et, al.,* 2002).Apelin was first isolated from bovine stomach extracts by (Tatemoto *, et, al.,* 1998); and (Tatemoto, *et, al.,* 1998). At least four forms of the peptide have been isolated, apelin-12, -13, -17 and -36 (Hosoya, *et, al.,* 2000); and (Szokodi, *et, al.,* 2002). Both apelin and APJ mRNA are highly expressed in the cardiovascular system in both humans and rats (Hosoya, *et, al.,* 2000); and (Szokodi, *et, al.,* 2002). There are similarities in amino acid sequence and tissue distribution with angiotensinogen and the angiotensin II receptor type 1 (AT_1). On the basis of these findings, several experimental studies have shown the physiological role of apelin in the cardiovascular system.

Apelin is the recently discovered angiotensin receptor like-1 (APJ), which potentially regulates heart function (Hosoya, *et, al.,* 2000). Apelin and APJ are widely expressed throughout the cardiovascular system (Lee, *et, al.,* 2000); and (Farkasfalvi, *et, al.,* 2007). Ex vivo as well as in vivo studies identified apelin as one of the potent inotropic substances (Szokodi, *et, al.,* 2002). Studies have shown that apelin has positive inotropic effects in vivo in both normal rat hearts and rat hearts in failure after myocardial infarction (Szokodi, *et, al.,* 2002); (Ashley, *et, al.,* 2005). (Szokodi *, et, al.,* 2002) used isolated perfused rat hearts,where infusion of apelin (0.01 to 10 nmol/L) induced a dose-dependent positive inotropic effect(Szokodi, *et, al.,* 2002). A plasma level of apelin has been shown to decrease in patients with heart failure (Kleinz and Davenport, 2005). Despite recent advances in the understanding of the cardiovascular effects of the apelin-APJ system, the myocardial protective effects of treating with apelin before resuscitation following hemorrhagic shock has not been investigated. The present study will investigate the myocardial protective effects of in vivo treatment with apelin before resuscitation following hemorrhagic shock in rats.

Materials and Methods

(1) Surgical Procedure

Male Sprague- Dawley rats were injected intra-peritonealy (i.p.) with heparin sodium 2000 I.U 15 minutes prior to anesthesia. The rats were then anaesthetized using urethane 125mg/kg intra-peritonealy. The left carotid artery was cannulated using polyethylene tubing size 60, and was connected to an in-line pressure transducer for continuous blood pressure monitoring. Animals were allowed to stabilize for a period of 30 minutes. The animals were assigned randomly to hemorrhagic treated, untreated, or similar time matched control groups (n = 6 per group).

(2) Hemorrhage and Resuscitation

Rats were hemorrhaged using a reservoir (a 10 ml syringe) that is connected to the arterial (carotid artery) three way stopcocks. Opening the stopcock and aspirating gently and gradually with the syringe induced hemorrhage. Blood was aspirated at a rate of 1 ml/min. Blood was continuously withdrawn or re-infused to the animal to maintain a mean arterial pressure of approximately 40 mmHg. The same surgical procedure were performed as for the sham hemorrhage group except rats were not be hemorrhaged. This group served as the time-matched control group to compare the data from the hemorrhage group. After 60 min hemorrhage period, hearts were resuscitated *in vivo*, by reinfusion of the shed blood to restore normotension and blood pressure were monitored for 30 min. Rats were either treated or not with intra-arterial injection of apelin-13.

(3) Harvest and Ex-vivo Perfusion of Hearts

Hearts were harvested and perfused ex-vivo for hemodynamic measurements using the Langendorff system. Hearts were perfused with normal physiological buffer KHB solution for 60 minutes. Sub-xiphoid, transverse incision was made in the abdomen and extended superiorly along both mid-axillary lines. The diaphragm was

carefully transected along its ventral margin and the ventral rib cage was lifted revealing the beating heart. Hearts were excised quickly and placed into ice physiologic saline to arrest the heart. Hearts were attached by the aorta to the proper size cannula in the Langendorff system. The hearts were perfused with non-circulating Krebs-Henseleit-Bicarbonate (KHB) buffer consisting of the following (in mM): sodium chloride, 118; calcium chloride, 1.25; potassium chloride, 4.7; sodium bicarbonate, 21; magnesium sulphate, 1.2; glucose, 11; potassium biphosphate, 1.2; and EDTA, 0.5. An apical stab incision was made in the left ventricle using a # 15 scalpel blade. A saline- filled cellophane balloon-tipped catheter was placed into the left ventricle (LV) via the mitral valve and was used to measure LV pressure and balloon volume. LV and perfusion pressures were measured using transducers placed at the levels of the heart and aorta. Hearts were stimulated electrically at 300 beats per minute using electrical stimulator (6020 Stimulator from Harvard Apparatus). Perfusion pressure was maintained at 50 mmHg. LVEDP was maintained at 5 mmHg. Perfusate temperature was maintained at 37^{o}C. The perfusate was gassed with a mixture of 95% O_2 +5% CO_2 at pH of 7.4 for the duration of the experiment.

(4) Experimental Groups

Three experimental groups (n=6) were assigned for the study:

(4.1) Normotensive Rats (N)

The same surgical preparation was performed. The rats were monitored for continuous blood pressure measurements for the experimental period 120 min.

(4.2) Hemorrhagic Shock Rats (HS)

After 30 min stabilization period, rats were hemorrhaged to 40 mmHg for 60 min. Rats were then resuscitated and monitored for 30 min.

(4.3) Effect of Apelin-13 during Hemorrhagic Shock (HS-AP)

After 30 min stabilization period, rats were hemorrhaged to 40 mmHg for 60 min. 1 ml of 10 nm /L apelin-13 was injected intra-arterially after the hemorrhage period and before resuscitation (Szokodi, Tavi *et, al.*, 2002). Rats were resuscitated and monitored for 30 min.

(5) Hemodynamic Measurements

At the end of the experimental period, hearts were harvested and perfused using the Langendorff Apparatus for 60 minutes. Myocardial function was determined with a balloon- tipped catheter inserted into the left ventricle via the mitral valve. Left ventricular end diastolic pressure (LVEDP), left ventricular peak systolic pressure (LVPSP), coronary perfusion pressure PP was recorded. The left ventricular ± dP/dt, which is the left ventricular index of contractility, was calculated.

(6) Statistical Analysis

(6.1) Data were initially analyzed with Bartlett's test for homogeneity.

(6.2) Data found not to be homogeneous were transformed and reanalyzed. Data were analyzed with multivariate analysis of variance (ANOVA).

(6.3) Means were analyzed using Duncan's test and were considered significant when yielding a "p" value less than or equal to 0.05.

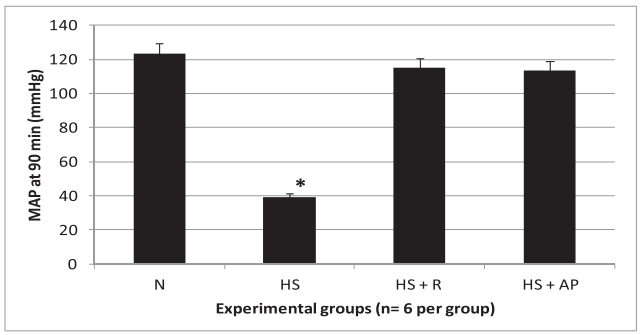
(6.4) Data were expressed as means ± SD.

Results

(1) The animals were subjected to hemorrhagic shock to lower the MABP to the desired level of hypotension (35-40 mmHg).

The total volume of blood withdrawn was 15 ± 1.5 mL/kg body weight. There was no significant difference in the amount of blood withdrawn among the groups of animals subjected to hemorrhagic shock.

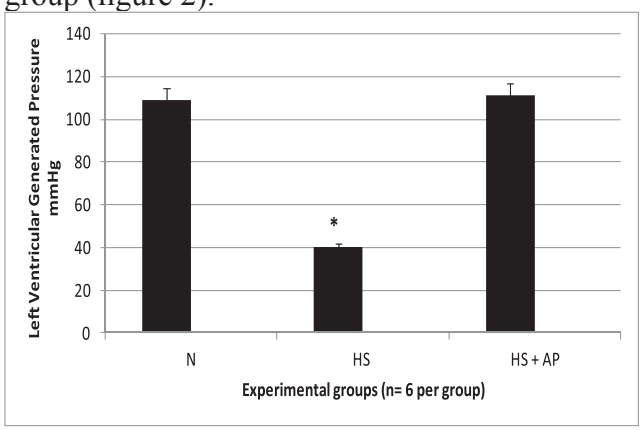
(2) Mean Arterial Blood Pressure: The normotensive rats maintained MABP at approximately 123.50 ± 3.4 mmHg, whereas the hemorrhagic shocked rats exhibited significantly lower levels($p < 0.05$) of MABP at approximately 39.2 ± 1.5 mmHg. In hemorrhagic shocked animals, intra-arterial administration of 1 ml of 10 nm /L apelin-13 did not cause a significant difference ($p > 0.05$) in the MABP at the end of the resuscitation compared with the normotensive (figure 1).



(**represents p< 0.05 between the resuscitated and non-resuscitated hemorrhagic shock groups*).

Figure 1: Effects of *in vivo* Treatment with AP on the MABP in Rats. Recording of the Arterial Blood Pressure after One-hour Hemorrhages and 30 Min of Resuscitation in the Normotensive Group (N), Hemorrhage Group Not Resuscitated (HS), Hemorrhage Group Resuscitated without AP (HS+R) and Hemorrhage Group Resuscitated with AP (HS-AP).

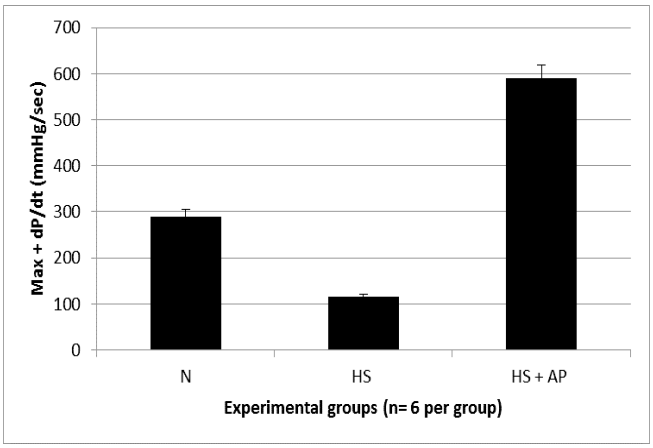
(3) Treatment with Apelin-13 Prevented Myocardial Contractile Dysfunction after Hemorrhagic Shock: The measurement of left ventricular generated pressure (LVGP) in the isolated hearts that was measured using the Langendorff system after treatment with AP and resuscitation of the hemorrhagic shocked animals showed significant increase ($p < 0.05$) in LVGP compared with the HS group (figure 2).



(**represents p< 0.05 versus hemorrhagic shock group, • represents p<0.05 versus the hemorrhagic shock group (n= 6 per group)*).

Figure 2: Effect of AP Treatment before Resuscitation of Hemorrhagic Shock on Left Ventricular Generated Pressure (LVGP) in the Normotensive (N), Hemorrhagic Shock (HS) and Hemorrhagic Shock+AP (HS-AP) (n= 6 per group). All the values are the means ± SD.

(4) Treatment with Apelin-13 Prevented Myocardial Contractile Dysfunction after Hemorrhagic Shock: The measurement of positive change in pressure over time (+dP/dt) in the isolated hearts that was calculated. Treatment with AP and resuscitation of the hemorrhagic shocked animals showed significant increase ($p< 0.05$) in +dP/dt compared with the HS group (figure 3).



*(All the values are the means ± SD. * represents p< 0.05 versus hemorrhagic shock group, • represents p<0.05 versus the hemorrhagic shock group (n= 6 per group)*).

Figure 3: Effect of AP Treatment Before Resuscitation of Hemorrhagic Shock on Positive Change in Pressure Over-time (+ dP/dt) (n= 6 per group).

The left ventricular generated pressure was significantly lower ($p < 0.05$) in the hemorrhagic shock group (40.0 ± 0.88 mmHg) compared with the normotensive group (109.15 ± 7.85 mmHg) (figure 2). The left ventricular generated pressure was significantly higher in animals treated with AP (111.20 ± 9.19 mmHg) than in hemorrhage non-treated animals ($p< 0.05$) (see, figure 2). The left ventricular +dP/dtmax was significantly lower ($p < 0.05$) in the hemorrhagic shock group (115.05 ± 35.7 mmHg/s) than in the normotensive group (289.83 ± 25.04 mmHg/s) (see, figure 3). The +dP/dtmax was significantly higher ($p < 0.05$) in the animals treated with AP (589.6 ± 110.68 mmHg) than in hemorrhage non-treated animals ($p< 0.05$) (see, figure 3).

Discussion

The present study demonstrated that treatment with apelin-13 before resuscitation of hemorrhagic shock protected the myocardial against post-resuscitation myocardial dysfunction. Apelin has been shown to have potent positive inotropic effects (Szokodi, *et, al.,* 2002). Animal and human studies suggest that apelin may play a role in the pathogenesis of heart failure by modulating the harmful effects of angiotensin II (Chandrasekaran, *et, al.,* 2008).This is in consistent with our results that demonstrated a significant positive inotropic effect of apelin in the hemorrhage treated group. (Berry*, et, al.,*2004) used native rats (n=32) and rats in heart failure 6 weeks after left anterior descending coronary artery ligation (n=22) with placement of a perivascular flow probe around the ascending aorta and a pressure volume conductance catheter into the left ventricle (Berry, *et, al.,* 2004).

The study showed that compared with sham-operated rats, the ligated rats had significantly decreased baseline Pmax and max dP/dt. Continous infusion of apelin at a rate of 0.01 μg/min for 20 minutes significantly increased max dP/dt in both native and failing hearts (Berry, *et, al.,* 2004) . The study also showed that apelin infusion increased cardiac contractility, indicated by a significant increase in stroke volume (Berry, *et, al.,* 2004). Another study used intraperitoneal injection of apelin (300 μg/kg) and used conductance catheter pressure-volume hemodynamic measurement and echocardiography in vivo (Ashley, *et, al.,* 2005).

The study showed that apelin increased contractile function and associate apelin with a positive hemodynamic profile and suggested that apelin is an attractive target for pharmacotherapy in heart failure. Another study used isolated adult ventricular myocytes to show the positive inotropic effects of apelin (Farkasfalvi, *et, al.,* 2007).

The study suggested that the positive inotropic effects of apelin is due to stimulation of the sarcolemmal Na-H exchanger, leading to intracellular alkalinization and increased myofilament sensitivity to Ca^{2+} (Farkasfalvi, *et, al.,* 2007).

The mechanism of the positive inotropic effect of apelin is not well understood. One possible explanation is that apelin lower the levels of the inflammatory mediators released following hemorrhagic shock (Sambol, *et, al.,* 2011). Apelin is known to have anti-inflammatory effects (Horiuchi, *et, al.,* 2003). Studies have shown that apelin can bind and activate its own receptors in the heart specifically, independent of release of endogenous angiotensin II, endothelin, catecholamine or nitric oxide (Szokodi, *et, al.,* 2002). (Szokodi, *et, al.,* 2002) also showed that activation of phospholipase C and protein kinase C (PKC) are involved in the positive inotropic effects of apelin (Szokodi, *et, al.,* 2002). Inhibition of sodium hydrogen exchange 1 (NHE-1) significantly attenuate ed the inotropic response to apelin, suggesting that activation of NHE , at least in part, contributes to the effect of apelin (Szokodi, *et, al.,* 2002).

The present study examined the protective effects of treatment with apelin before resuscitation of hemorrhagic shock in a rat model. Further studies are needed to examine the effects of treatment with apelin in vivo. The results may open a new field of treatment of hemorrhagic shock and improve the overall outcome in patients with hemorrhagic shock.

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References

Ashley EA; Powers J; Chen M; Kundu R; Finsterbach T; Caffarelli A; Deng A; Eichhorn J; Mahajan R; Agrawal R; Greve J; Robbins R; Patterson AJ; Bernstein D; and **Quertermous T** (2005) The Endogenous Peptide Apelin Potently Improves Cardiac Contractility and Reduces Cardiac Loading In-vivo. *Cardiovascular Research,* **65** (1): 73-82.
Available at: http://www.cardiovascres.oxfordjournals.org/content/65/1/73.full

Berry MF; Pirolli TJ; Jayasankar V; Burdick J; Morine KJ; Gardner TJ; and **Woo YJ** (2004) Apelin has In-vivo Inotropic Effects on Normal and Failing Hearts. Circulation, **110** (11 Suppl 1): II187- II193.
Available at:http://www.circ.ahajournals.org/content/110/11_suppl_1/II-187/F6.expansion.html

Chandrasekaran B; Dar O; and **McDonagh T** (2008) The Role of Apelin in Cardiovascular Function and Heart Failure. *European Journal of Heart Failure,* **10** (8): 725-732.
Available at:http://www.eurjhf.oxfordjournals.org/content/10/8/725.full.pdf

Farkasfalvi K; Stagg MA; Coppen SR; Siedlecka U; Lee J; Soppa GK; **Marczin N; Szokodi I; Yacoub MH**; and **Terracciano CM** (2007) Direct Effects of Apelin on Cardiomyocyte Contractility and Electrophysiology. *Biochemical and Biophysical Research Communications,* **357** (4): 889-895.
Available at: http://www.sciencedirect.com/science/article/pii/S0006291X07007152
Available at: http://www.ncbi.nlm.nih.gov/pubmed/17466269

Horiuchi Y; Fujii T; Kamimura Y; and **Kawashima K** (2003) The Endogenous Immunologically Active Peptide Apelin Inhibits Lymphocytic Cholinergic Activity during Immunological Responses. *Journal of Neuroimmunology,* **144** (1/2): 46-52.
Available at:http://www.ncbi.nlm.nih.gov/pubmed/14597097

Hosoya M; Kawamata Y; Fukusumi S; Fujii R; Habata Y; Hinuma S; Kitada C; Honda S; Kurokawa T; Onda H; Nishimura O; and **Fujino M** (2000) Molecular and Functional Characteristics of APJ. Tissue Distribution of mRNA and Interaction with the Endogenous Ligand Apelin. *The Journal of Biological Chemistry,* **275** (28): 21061-21067.
Available at: http://www.ncbi.nlm.nih.gov/pubmed/10777510

Kleinz MJ; and **Davenport AP** (2005) Emerging Roles of Apelin in Biology and Medicine. *Pharmacology and Therapeutics.*, **107** (2): 198-211.
Available at: http://www.ncbi.nlm.nih.gov/pubmed/15907343

Kline JA; Thornton LR; Lopaschuk GD; Barbee RW; and **Watts JA** (1999) Heart Function after Severe Hemorrhagic Shock. *Shock (Augusta Ga),* **12** (6): 454-456.
Available at: http://www.ncbi.nlm.nih.gov/pubmed/10588514

Lee DK; Cheng R; Nguyen T; Fan T; Kariyawasam AP; Liu Y; Osmond DH.; George SR; and **O'Dowd BF** (2000) Characterization of Apelin; the Ligand for the APJ Receptor. *Journal of Neurochemistry,* **74** (1): 34-41.
Available at: http://www.ncbi.nlm.nih.gov/pubmed/10617103

Sambol JT; Lee MA.; Jiang M; Dosi G; Dong W; Deitch EA; and **Yatani A** (2011) Mesenteric Lymph from Rats with Trauma Hemorrhagic Shock Causes Abnormal Cardiac Myocyte Function and Induces Myocardial Contractile Dysfunction. *Journal of Applied Physiology (Bethesda Md.:1985)*, **111** (3): 799-807.
Available at: http://www.ncbi.nlm.nih.gov/pubmed/21700891

Szokodi I; Tavi P; Földes G; Voutilainen Myllylä S; Ilves M; Tokola H; Pikkarainen S; Piuhola J; Rysä J; Tóth M; and **Ruskoaho H** (2002) Apelin; the Novel Endogenous Ligand of the Orphan Receptor APJ; Regulates Cardiac Contractility. *Circulation Research,* **91** (5): 434-440.
Available at: http://www.ncbi.nlm.nih.gov/pubmed/12215493

Tatemoto K; Hosoya M; Habata Y; Fujii R; Kakegawa T; Zou MX.; Kawamata Y; Fukusumi S; Hinuma S; Kitada C; Kurokawa T; Onda H; and **Fujino M** (1998) Isolation and Characterization of a Novel Endogenous Peptide Ligand for the Human APJ Receptor. *Biochemical and Biophysical Research Communications*, **251** (2): 471-476.
Available at: http://www.ncbi.nlm.nih.gov/pubmed/9792798