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Modulation by atrial natriuretic factor of receptor-mediated cyclic AMP-dependent responses in canine pulmonary artery during heart failure

[1,*]Rajamma Mathew, Hatim A. Omar, Raisa Fayngersh, Weiqun Shen, Jie Wang, *Michael H. Gewitz, Thomas H. Hintze & Michael S. Wolin

Departments of *Paediatrics and Physiology, New York Medical College, Valhalla, New York, 10595, U.S.A.

1 Pacing-induced congestive heart failure (CHF) in dogs is associated with increased plasma levels of atrial natriuretic factor (ANF) and inhibiton of receptor-mediated cyclic AMP-dependent relaxation in isolated pulmonary arteries (PA). Since ANF is known to be negatively coupled to adenylate cyclase, we studied cyclic AMP-mediated relaxation to isoprenaline (Iso) and arachidonic acid (AA) in PA from control dogs (C), dogs with pacing-induced CHF (CHF) and dogs with bilateral atrial appendectomy and CHF (ATR APP+CHF).

2 In CHF, plasma ANF levels increased from a baseline of 80 ± 8 pg ml^{-1} to 283 ± 64 pg ml^{-1} ($P<0.05$), but the ATR APP+CHF group failed to show this increase (67 ± 7 pg ml^{-1} vs 94 ± 15 pg ml^{-1}, P=NS). Plasma ANF levels, however, did not influence myocardial dysfunction in CHF.

3 The relaxation of $49 \pm 5\%$ to 1 μM Iso in C was reduced to $23 \pm 4\%$ in CHF ($P<0.05$), but relaxation of $49 \pm 12\%$ was observed in the ATR APP+CHF group (P=NS vs C). Relaxation responses to 10 μM AA were as follows: $77 \pm 5\%$ (C, $n=8$), $27 \pm 8\%$ (CHF, $n=10$, $P<0.05$ vs C), and $93 \pm 5\%$ (ATR APP+CHF, $n=5$). The presence of CHF, or the plasma ANF levels, did not affect responses to cyclic GMP-mediated relaxing agents in PA.

4 These data indicate that the myocardial performance in CHF is not influenced by plasma ANF levels. However, altered cyclic AMP-mediated relaxation in PA during CHF is, in part, modulated by circulating ANF levels.

Keywords: Adenylate cyclase; arachidonic acid; atrial natriuretic factor; isoprenaline; G proteins; pulmonary vascular reactivity

Introduction

Congestive heart failure (CHF) is associated with high levels of circulating atrial natriuretic factor (ANF) (Burnett *et al.*, 1986), and elevated plasma ANF levels are correlated with a poor prognosis (Gottlieb *et al.*, 1989). ANF is a peptide hormone involved in the regulation of blood volume and pressure (DeBold 1985; Genest & Cantin, 1987). It is normally secreted by atria in response to stretch, and bilateral atrial appendectomy eliminates ANF release during volume loading in conscious dogs (Edwards *et al.*, 1988; Stewart *et al.*, 1992). ANF has been shown to inhibit adenylate cyclase activity, and ANF clearance receptors are coupled to the adenylate cyclase /adenosine 3':5'-cyclic monophosphate (cyclic AMP) system via a pertussis toxin sensitive mechanism (Anand-Srivastava *et al.*, 1990). Our recent studies reveal that the pacing-induced heart failure in dogs is associated with marked attenuation of receptor-mediated cyclic AMP-dependent relaxation responses in isolated pulmonary arteries (Mathew *et al.*, 1993). Since the atrial appendages are the primary source of ANF, and ANF is negatively coupled to the adenylate cyclase/cyclic AMP signal transduction mechanism, we hypothesized that elevated plasma ANF levels during CHF would have a significant effect on vascular reactivity of isolated canine pulmonary artery during CHF. Secondly, we speculated that if we prevented the increase in plasma ANF levels during the development of heart failure, we would prevent the inhibition of receptor-mediated cyclic AMP-dependent relaxation responses. Therefore, we examined cyclic AMP-mediated relaxation responses to isoprenaline (Iso) and arachidonic acid in isolated pulmonary arteries from control dogs, dogs with pacing-induced CHF, and from a group of dogs with bilateral atrial appendectomy and CHF (ATR APP+CHF). Included in this study were evaluations of contractile responses to phenylephrine and guanosine 3':5'-cyclic monophosphate (cyclic GMP)-mediated relaxation responses to acetylcholine and bradykinin. Plasma ANF levels and haemodynamic parameters were also obtained from these groups of animals.

[1] Author for correspondence at: Section of Pediatric Cardiology, New York Medical College, Munger Pavilion, Valhalla, NY 10595, U.S.A.

Methods

Mongrel dogs weighing 18–25 kg were allowed Purina laboratory canine chow and water *ad libitum*.

Procedure for atrial appendectomy

On the day of initial experiment, the dogs were sedated with acepromazine (3 mg kg^{-1}, s.c.), anaesthetized with sodium pentobarbitone (25 mg kg^{-1}, s.c.) and placed on a positive pressure ventilator (Harvard Apparatus, South Natick, MA). They were instrumented with a femoral artery catheter which was tunneled subcutaneously to the neck. An incision was made in the fourth left intercostal space and the chest opened. The right and left atrial appendages were clamped, sutured behind the clamp and excised in entirety following a method described previously (Steward *et al.*, 1992). For the control group, the dogs underwent similar instrumentation without atrial appendectomy (sham operated group). The dogs were allowed to recover for 14 days before the induction of heart failure.

Procedure for pacing-induced CHF

Pacing-induced heart failure was produced in both sham-operated (CHF group) and atrial appendectomy operated (ATR APP+CHF) dogs in the manner described previously (Mathew *et al.*, 1993). Briefly, the dogs were anaesthetized, through a thoracotomy by sterile techniques, a Tygon catheter was placed in the descending thoracic aorta and a solid state pressure gauge (Konigsberg P6.5, Pasedena, CA) was placed in the left ventricle through the apex. A corkscrew electrode was placed in the left ventricle attached to a portable external pacemaker (Pace Medical EV3434, Waltham, MA). The chest was closed and the dogs allowed to recover fully from anaesthesia before returning to their respective cages.

The dogs were allowed to recover from surgery, and 7–10 days later, when they were afebrile and had been trained to lie quietly without restraint on the laboratory table, baseline haemodynamic data and blood for ANF levels were obtained. After control responses were obtained, each dog began a regimen of continuous ventricular pacing. A pacing rate of 210 beats min^{-1} was maintained for 3 weeks, then the rate was increased to 240 beats min^{-1} for an additional one week. Haemodynamic studies were repeated after the development of CHF with the pacemaker turned off for two hours. Haemodynamic parameters included the measurements of heart rate, aortic pressure, left atrial pressure, left ventrical and diastolic pressure and the first derivative of left ventricular pressure with time (an index of ventricular contractility, LV *dp/dt*, expressed as mmHg s^{-1}). Cardiac output was estimated by multiplying heart rate by the calculated left ventricular volume. Blood samples were also obtained for plasma ANF measurement. At the end of the study the dog was given a lethal dose of pentobarbitone and through a thoracotomy the lungs were removed with care and placed in cold Krebs buffer for the isolated pulmonary artery studies.

Plasma ANF levels

The radioimmunoassay for ANF was performed by a previously described method (Hintze *et al.*, 1989). Briefly, blood was taken from the aortic catheter and placed in a chilled tube containing EDTA and 5 $\mu l\ ml^{-1}$ aprotinin. The samples were centrifuged and the plasma was stored at −20°C. On the day of analysis the samples were thawed, extracted by use of a SEP-PAK C-18 cartridge and ANF was determined with a commercially available RIA kit (Penisula Laboratories, Belmont, CA).

Isolated pulmonary artery study

The pulmonary vascular reactivity studies from sham-operated control dogs and unoperated dogs were similar, therefore, in the control (C) group we used lungs from both types of animals. Second and third order branches of lobar pulmonary arteries were dissected and cleaned, taking care not to damage the endothelium, and 3–4 mm wide rings were prepared. In some rings, endothelium was deliberately removed by gentle rubbing of the luminal surface with a tapered wooden stick. Rings were mounted on wire hooks attached to a Grass (FT03) force displacement transducer at a basal tension of 6 g and changes in isometric forces were recorded on a Grass polygraph (model 7). Rings were allowed to equilibrate for one hour in Krebs bicarbonate buffer (composition in mM): NaCl 118, KCl 4.7, $CaCl_2$ 1.5, $NaHCO_3$ 25, $MgSO_4$ 1.1, KH_2PO_4 1.5, glucose 5.6) in individual 10 ml organ baths (Metro Scientific, Long Island, NY), maintained at 37°C and aerated with 95%O_2/5%CO_2 (pH 7.4). The rings were allowed to equilibrate in drug free Krebs buffer for 30 min between experimental cycles. Data were pooled for each type of vessel in each animal and an average was calculated. Since acetylcholine, arachidonic acid and bradykinin require intact endothelium, we analyzed endothelium-intact arteries for all groups.

In this study, the resting tension is defined as 0, and measurements are presented as active force generation above the baseline. The relaxation response is expressed as % decrease in tone in relation to the initial tone induced by phenylephrine.

Evaluation of contractile response

Arterial rings from all groups were depolarized with 60 mM KCl for 5 min. Following a 30 min equilibration in drug free Krebs buffer, a concentration-response to phenylephrine (PE, 10 nM–1 μM) was obtained in all rings. For subsequent experiments a concentration of PE (100 nM–500 nM) that produced 50–60% of maximal contraction was used.

Evaluation of endothelium-dependent cyclic GMP-mediated responses

Following the PE concentration-response curve, once a steady state was reached, a concentration-response curve to acetylcholine (10 nM–10 μM) was obtained to assess the endothelium-dependent relaxation response. Similarly a concentration-response curve to bradykinin (1 nM–1 μM) was obtained on arterial rings precontracted with PE.

Evaluation of receptor-mediated cyclic AMP-dependent relaxation responses

The arterial rings were precontracted with PE as described earlier and relaxation responses to isoprenaline (1 nM–1 μM) and arachidonic acid (10 nM–10 μM) were obtained.

Drugs

All chemicals were obtained from Sigma Chemical Co (St. Louis, MO). Phenylephrine HCl, isoprenaline HCl, acetylcholine chloride and bradykinin acetate were dissolved in deionized water. Arachidonic acid (AA) was dissolved in 100 mM Na_2CO_3 and deionized water to obtain a final solution of 10 mM in 10 mM Na_2CO_3, and was collected in small aliquots under nitrogen and stored at −80°C. Further dilutions

Table 1 Haemodynamic parameters and plasma atrial natriuretic factor (ANF) levels before and after the onset of congestive heart failure (CHF)

	Sham operated group		*Atrial appendectomy group*	
	Before	*After*	*Before*	*After*
Heart rate (beats min^{-1})	100±7	123±8*	105±6	143±3*
MAP (mmHg)	99±3	92±4*	97±3	86±3*
LAP (mmHg)	4.5±1	12±1.4*	5.5±0.9	14.6±3*
LVEDP (mmHg)	7±1.2	13.9±2*	7±1.3	13±1.7*
LV *dp/dt* (mmHg s^{-1})	2994±167	2026±192*	2722±185	1775±62*
Plasma ANF (pg ml^{-1})	80±8	283±64*	67±7	94±15

Haemodynamic parameters were obtained in the same group of animals before and after the onset of congestive heart failure (n=4–10). MAP=mean aortic pressure, LAP=left atrial pressure, LVEDP=left ventricular end diastolic pressure, LV *dp/dt*=first derivative of LV pressure with time, *$P<0.05$ vs before the onset of CHF.

were made just before use. During the experiments the drug solutions were kept on ice.

Statistical analysis

The results are expressed as mean ± s.e. Analysis of variance for multiple responses by use of Scheffe's test was performed. A P value of <0.05 was considered statistically significant.

Results

Clinical signs of heart failure such a tachycardia (with the pacer off), tachypnoea, ascites, pleural effusion and impaired myocardial function were present in both groups of heart failure. In the CHF group, the plasma ANF levels were significantly increased after the onset of heart failure, whereas this increase was not observed in the ATR APP + CHF group. The basal level of ANF, however, was not altered by atrial appendectomy alone (Table 1). As shown in Table 1, myocardial function was depressed to a similar extent in both groups of heart failure. Estimated cardiac output was not different in the heart failure groups (data not shown). The plasma ANF levels did not influence myocardial function in heart failure.

Contractile responses

As shown in Figure 1a, the contractile response to PE was not significantly different in any of the groups.

Cyclic GMP-mediated relaxation responses

Bradykinin induced endothelium-dependent relaxation response in pulmonary arteries from all the groups studied. In an analogous fashion to bradykinin, there were no significant differences in relaxation responses to acetylcholine in any of the groups (Figure 1b and c).

Receptor-mediated cyclic AMP-dependent relaxation responses

Response to Isoprenaline As shown in Figure 2, the pulmonary arteries from the CHF group showed significant attenuation of relaxation responses to higher concentrations of Iso (10 nM and 1 μM) compared with the control group ($P<0.05$). The pulmonary arterial rings from the ATR APP + CHF group exhibited normal relaxation responses to (1 μM) Iso ($P<0.05$ vs CHF group).

Response to arachidonic acid The relaxation response to AA (1 μM – 10 μM) was depressed in the CHF group compared with the control group ($P<0.05$). As shown in Figure 3, the ATP APP + CHF group showed improved relaxation at concentrations of 1 μM – 10 μM ($P<0.05$ vs CHF), and this response was not significantly different when compared with control. At lower concentrations of AA (10 nM – 100 nM), ATR APP + CHF group demonstrated significantly increased relaxation compared with control and CHF groups ($P<0.05$).

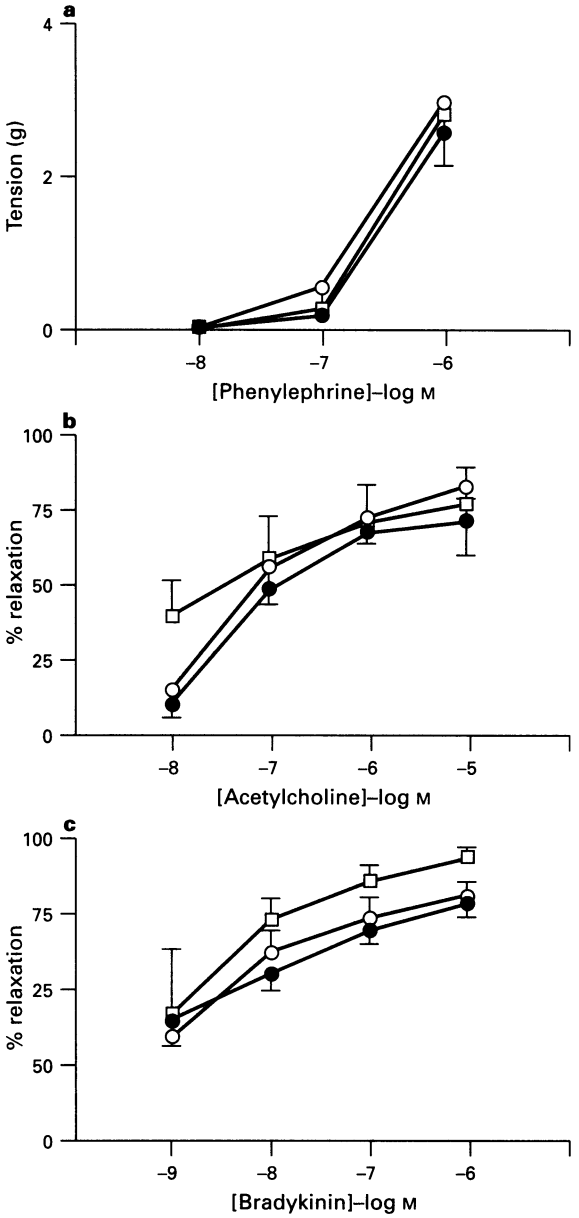

Figure 1 The peak tone reached at different concentrations of phenylephrine during the concentration-response study in canine isolated pulmonary arteries from control (○, $n=7$), congestive heart failure (CHF, ●, $n=7$) and bilateral atrial appendectomy + CHF (ATR APP + CHF, □, $n=5$) groups (a). The relaxation responses (calculated as % decrease of the phenylephrine-induced contraction) to cumulative concentrations of acetylcholine (b, control $n=7$, CHF $n=7$, ATR APP + CHF $n=4$) and bradykinin (c, n same as for acetylcholine) in isolated pulmonary arteries are also shown. Note, there were no significant differences between the contractile response to phenylephrine or cyclic GMP-mediated relaxation responses to acetylcholine and bradykinin in any of the groups.

Discussion

The present study indicates a potential relationship between plasma ANF levels and cyclic AMP-mediated relaxation responses in canine isolated pulmonary arteries following pacing-induced CHF. In this model of CHF, increased synthesis and release of ANF from atria have been well documented (Perella *et al.*, 1992). The increase in plasma ANF levels was absent in ATR APP + CHF group confirming that the atrial appendages contribute to the increase in plasma ANF levels during pacing-induced heart failure. This failure to increase plasma ANF levels in ATR APP + CHF group was associated with improved cyclic AMP-mediated relaxation to Iso and AA in the isolated pulmonary arteries. However, neither heart failure nor the plasma ANF levels influenced the contractile response to phenylephrine or cyclic GMP-mediated relaxation responses. In the ATR APP + CHF group, the clinical signs and myocardial dysfunction associated with heart failure were not altered. Left atrial pressure (LAP) and left ventricular end diastolic pressure (LVEDP) in both the heart failure groups were elevated to similar levels. Since LAP and LVEDP influence the pulmonary artery pressure, the effects on pulmonary artery pressure would be similar in both of the failure groups. Thus, the observed differences in cyclic AMP-mediated relaxation cannot be attributed to haemodynamic factors.

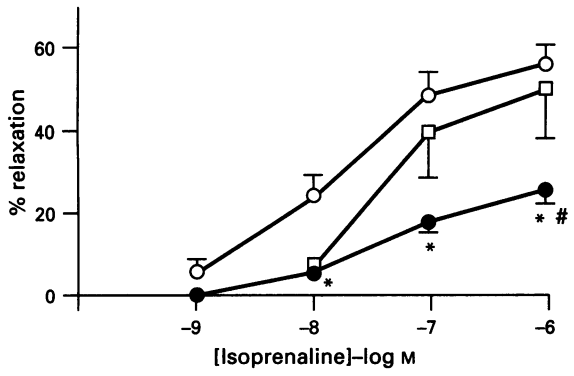

Figure 2 The relaxation response to cumulative concentrations of isoprenaline expressed as % decrease in phenylephrine-induced tone in canine isolated pulmonary arteries from the control group (○, $n=13$), the congestive heart failure (CHF) group (●, $n=12$) and the bilateral atrial appendectomy+CHF (ATR APP+CHF, □, $n=5$) group. Note, there was significant inhibition of the relaxation response in the CHF group, but the ATR APP+CHF group showed normal relaxation responses. $^{*}P<0.05$ vs control, $^{\#}P<0.05$ vs ATR APP+CHF.

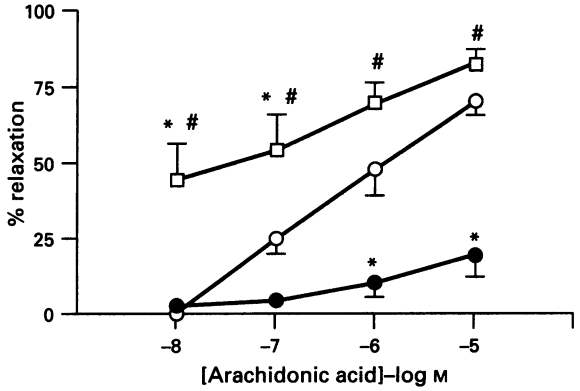

Figure 3 The relaxation response to cumulative concentrations of arachidonic acid (AA) expressed as % decrease from the tone elicited by phenylephrine in canine isolated pulmonary arteries from the control group (○, $n=8$), the congestive heart failure (CHF) group (●, $n=10$) and the bilateral atrial appendectomy+CHF (ATR APP+CHF, □, $n=5$) group. Note, in the CHF group, the relaxation to 10^{-6}–10^{-5} M AA was significantly reduced compared with the control group, whereas in the ATR APP+CHF group, the relaxation to the same concentrations of AA was normal. However, the relaxation to lower concentrations of AA significantly increased in the ATR APP+CHF group compared with the control group. $^{*}P<0.05$ vs control, $^{\#}P<0.05$ vs CHF.

Recently, we found that the cyclic AMP signal transduction mechanism remained normal in pulmonary arteries in the absence of overt signs of heart failure, despite myocardial dysfunction (Mathew *et al.*, 1993). In this context, it is worth noting that the basal plasma ANF levels and haemodynamic parameters were similar in the sham-operated control group and the group with atrial appendectomy before the onset of CHF. Thus, the atrial appendectomy procedure itself does not affect basal ANF levels or myocardial function. Therefore, it is unlikely that under basal conditions, atrial appendectomy could alter the pulmonary circulation or cyclic AMP signal transduction mechanism in pulmonary arteries.

Iso and prostaglandin I_2(PGI_2), a metabolite of AA, via β-adrenoceptors and PGI_2 specific receptors respectively, are coupled to the activation of adenylate cyclase via stimulating guanine nucleotide binding proteins (G_s), in which increase intracellular cyclic AMP leading to smooth muscle relaxation (Johnson *et al.*, 1985; Hashimoto *et al.*, 1990). In the present study, isolated pulmonary arteries from dogs with ATR APP+CHF, show a normal relaxation response to high concentrations of Iso (1 μM) and AA (1 μM–10 μM), whereas, a significant inhibition of these relaxation responses was seen in the CHF group with increased plasma ANF levels. Although both Iso and AA induced receptor-mediated cyclic AMP-dependent relaxation, the responses to low concentrations of Iso and AA in pulmonary arteries from ATR APP+CHF group were quite different. At low concentrations, the relaxation response to AA was enhanced, whereas, the response to Iso was attenuated. The reason for these differences is not clear. Since, both Iso and AA induce cyclic AMP-mediated relaxation via different receptors, it is possible that the heart failure affects these receptors differently.

There are several possibilities as to how increased levels of ANF might inhibit cyclic AMP-mediated relaxation responses. ANF has been shown to inhibit basal and stimulated adenylate cyclase activity and cyclic AMP generation in a number of tissues including vascular smooth muscle via a pertussis toxin sensitive mechanism (Resnick *et al.*, 1988; Anand-Srivastava *et al.*, 1990). C-ANF a peptide that binds to clearance receptors (Maack *et al.*, 1987), is known to inhibit adenylate cyclase activity in different tissues (Anand-Srivastava *et al.*, 1984; Drewett *et al.*, 1992). These data indicate the involvement of G_i in the process of ANF-induced inhibition of adenylate cyclase. In some tissues such as adrenal glomerulosa cells and human fibroblasts, ANF inhibits cyclic AMP via stimulation of cyclic GMP-stimulated phosphodiesterase. This mechanism is G_i-independent (Lee *et al.*, 1988; McFarland *et al.*, 1991). However, it is not known whether this mechanism plays a role in the inhibition of adenylate cyclase activity in vascular tissue. Further studies are required to clarify these possibilities. It is interesting to note that during CHF, myocardium exhibits a depressed inotropic response to Iso and an increased activity of G_i (Feldman *et al.*, 1988; Belloni *et al.*, 1992).

Increased circulating catecholamines and prostaglandins are known to be present in CHF (Dzau *et al.*, 1984; O'Brien *et al.*, 1990), which could down regulate their respective receptors and alter cyclic AMP-mediated responses. However, ANF has an inhibitory effect on sympathetic activity and on stimulated catecholamine synthesis by a pertussis toxin-sensitive mechanism suggesting an involvement of G_i (Debinski *et al.*, 1987; Drewett *et al.*, 1992). ANF is not considered to have any influence on PGI_2 release (Griesmaher *et al.*, 1989). Therefore, it is unlikely that the absence of an increase in plasma ANF levels during CHF in the ATR APP+CHF group could have a major effect on circulating catecholamines or prostaglandins to account for the altered cyclic AMP-mediated responses.

In summary, our data indicate that the atrial appendages contribute to the increase in plasma ANF levels in pacing-induced heart failures in dogs. Plasma ANF levels do not affect myocardial dysfunction observed in heart failure. The contractile response to phenylephrine and endothelium-dependent cyclic GMP-mediated relaxation responses are not affected by CHF or by plasma ANF levels. However, cyclic AMP-mediated relaxation in isolated pulmonary arteries during heart failure, is significantly attenuated in the presence of increased circulating levels of ANF. In the absence of an increase in plasma ANF levels in the ATR APP+CHF group, relaxation to higher concentrations of Iso and AA in pulmonary arteries are normal. These observations indicate that the plasma ANF levels modulate in part, cyclic AMP-mediated relaxing mechanisms in the canine pulmonary arteries during CHF.

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