Plasma atrial natriuretic peptide concentrations in horses with heart disease: A pilot study

Trachsel, D; Grenacher, B; Weishaupt, M A; Schwarzwald, C C

Abstract: Atrial natriuretic peptide (ANP) is a cardiovascular biomarker that might be useful in assessing the severity of cardiac disease in horses. Plasma ANP concentrations (Cp(ANP)) were compared between horses with heart disease but normal chamber size and function (Group A; n=6), horses with heart disease associated with left atrial (LA) enlargement, LA dysfunction, and/or left ventricular (LV) enlargement (Group B; n=5), and horses with no clinically apparent cardiovascular disease (Group C; n=13). The median (min-max) for Cp(ANP) was significantly higher in Group B (53.5 (36.0-70.7) pg/mL), compared to Group A (12.5 (6.3-19.8) pg/mL) and Group C (13.4 (7.2-34.0) pg/mL). Backwards stepwise multiple linear regression showed that Cp(ANP) in horses with heart disease was related to LA dimensions, but not to LV size, LA function, and LV function. The results indicated that Cp(ANP) in horses might be useful in detecting LA enlargement and that Cp(ANP) could be related to the severity of cardiac disease. Larger prospective studies are necessary to confirm these results.

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Plasma atrial natriuretic peptide concentrations in horses with heart disease: A pilot study

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A B S T R A C T

Atrial natriuretic peptide (ANP) is a cardiovascular biomarker that might be useful in assessing the severity of cardiac disease in horses. Plasma ANP concentrations (CpANP) were compared between horses with heart disease but normal chamber size and function (Group B; n = 6), horses with no clinically apparent cardiovascular disease (Group A; n = 13), and horses with left atrial (LA) enlargement, LA dysfunction, and/or left ventricular (LV) enlargement (Group C; n = 13).

The median (min–max) for CpANP was significantly higher in Group B (53.5 (36.0–70.7) pg/mL), compared to Group A (12.5 (6.3–19.8) pg/mL) and Group C (13.4 (7.2–34.0) pg/mL). Backwards stepwise multiple linear regression showed that CpANP in horses with heart disease was related to LA dimensions, but not to LV size, LA function, and LV function. The results indicated that CpANP in horses might be useful in detecting LA enlargement and that CpANP could be related to the severity of cardiac disease. Larger prospective studies are necessary to confirm these results.

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Introduction

Natriuretic peptides are widely used as cardiac biomarkers in human and veterinary medicine (van Kimmenade and Januzzi, 2009). Physiologically, they counteract the effects of the renin–angiotensin–aldosterone system and regulate fluid balance and blood pressure by a variety of renal, vascular, and cardiac mechanisms (McGrath et al., 2005). Natriuretic peptides are produced by atrial and ventricular myocytes and released into the blood in response to a variety of triggers.

The plasma concentration of brain (B-type) natriuretic peptide (BNP; a peptide more abundant in ventricular myocytes) and amino-terminal proBNP (NT-proBNP; the more stable, inactive remnant that is released when BNP is activated from its prohormone proBNP) are most widely used in human medicine, particularly to diagnose diseases associated with ventricular dysfunction and heart failure (Daniels and Maisel, 2007). However, measurement of plasma BNP and NT-proBNP concentrations requires species-specific assays, since the amino acid sequence of BNP varies among species (Liu et al., 2002). To date, an assay to measure equine BNP or NT-proBNP concentrations is not commercially available.

Conversely, the amino acid sequence of atrial natriuretic peptide (ANP) is highly conserved among species, allowing the use of commercially available human assays to measure plasma ANP concentrations (CpANP) in horses (Richter et al., 1998). ANP is primarily produced by atrial myocytes, although its expression can also be enhanced in diseased ventricular myocytes from experimental animals and in human patients (Ruskoaho, 2003). In addition to atrial stretch or increased pacing frequency, ANP expression and secretion can be increased by a variety of stimuli, including endothelin-1, arginine vasopressin, angiotensin II, α-adrenergic agonists, thyroid hormones, glucocorticoids, growth hormone, prostaglandins, vitamin D, thrombin (de Bold et al., 1996; Ruskoaho, 2003) and hypoxia (Chen, 2005). The increased ANP secretion following atrial myocardial stretch due to volume or pressure overload is certainly of particular interest in equine cardiology, since horses frequently suffer from mitral regurgitation (MR) or atrial fibrillation (AF), which both affect atrial size and mechanical function.

Storage of ANP in the equine atrial myocytes and its release during stretching of atrial tissue (as occurring during exercise) has been documented in healthy horses (Mifune et al., 1991; Kokkonen et al., 1995). More recently, it has been shown that CpANP may be slightly elevated in horses with valvular regurgitation associated with left atrial (LA) enlargement or combined LA and left ventricular (LV) dilation, compared to healthy horses (Gehlen et al., 2007). However, the use of ANP as a cardiac biomarker has not yet been
fully investigated in horses. It is unknown to what extent CPANP increases with various heart diseases and whether the measurement of CPANP is of any diagnostic or prognostic value in horses.

The aim of this study was to measure CPANP in a group of horses suffering from various heart diseases and to investigate the association between CPANP and echocardiographic measurements of chamber dimensions and chamber function. We hypothesised that horses with cardiac disease associated with LA enlargement, LA dysfunction, and/or LV enlargement would have increased CPANP compared to horses with cardiac disease and normal LA and LV size and function and to horses without clinically apparent heart disease.

Material and methods

Study population

The main study population consisted of 11 horses that were presented to the Equine Hospital of the Vetsuisse Faculty of the University of Zurich for cardiovascular examination because of existing heart disease (Groups A and B). The population consisted of one female, seven castrated males, and three intact males of various breeds (six Warmbloods, two Arabians, one Thoroughbred, one Friesian, one Paso Fino) with a median age of 14 years (range 3–29 years) and a bodyweight (BW) of 530 kg (range 360–600 kg).

Another group of 13 horses served as control group (Group C). This group comprised six females, six castrated males, and one intact male of various breeds (six Warmbloods, two Icelandic horses, one Appaloosa, one Palomino, one Zangershede, one Shire, one Freiberger) with a median age of 8 years (range 3–19 years) and a median BW of 510 kg (range 400–690 kg; n = 9).

All examinations, including physical examination, and collection of blood samples, were performed within the routine patient workup and therefore did not require approval by the district veterinary office. Owners’ consent was obtained.

Echocardiographic examination

All horses of group A and B underwent a thorough cardiologic examination, including physical examination, thoracic and cardiac auscultation, electrocardiography, and standardised thoracosternal two-dimensional (2DE) and M-mode echocardiography (Boon, 1998; Bonagura et al., 2010). Echocardiographic recordings (GE Vivid 7 Dimension (BTO6) with a M4S Phased Array Transducer, GE Healthcare) were obtained by three trained and experienced clinicians within the routine workup of the cases. All measurements were performed offline from the digitally stored cine loop recordings by one single operator (DST) for the purpose of this study.

The echocardiographic examination included conventional 2DE and M-mode measurements of LV dimensions and LV systolic function (Boon, 1998; Bonagura et al., 2010). The motion of LA and LV mechanical function using previously described imaging standards (Schwarzwald et al., 2007; Bonagura et al., 2010) and/or published in the literature (Boon, 1998; Schwarzwald et al., 2007; Bonagura et al., 2010) and/or established in our echocardiography laboratory. During echocardiographic recordings, offline analyses, and final assessment, the observers were blinded to the results of the CPANP measurements.

Signalment, diagnoses, and echocardiographic indices of all horses are listed in Table 1. Six horses had normal LA and LV dimensions and mechanical function by echocardiography (Group A). One of the horses had MR, two had aortic regurgitation (AR), one had AR and a restrictive ventricular septal defect (VSD), one showed both AR and MR, and one showed MR, AR, and traces of tricuspid regurgitation (TR) and pulmonic regurgitation (PR). Five horses suffered from cardiac disease associated with LA enlargement, LA dysfunction, and/or LV enlargement (Group B). In this group, one horse had LA dysfunction associated with chronic AF (lasting at least 1 year) without concomitant valvular diseases and without LA and LV enlargement, one horse had moderate to severe valvular regurgitation to mild MR with an enlarged but normal LA size and function, and three horses had increased LV dimensions concomitant to LA enlargement and/or LA dysfunction, associated with multiple valvular regurgitations, AR, and aorto-cardiac fistula, respectively. Two horses in Group B showed signs of congestive heart failure (CHF).

Blood sampling

The very short half-life-time (estimated to be approximately 2–5 min in sheep and humans (Bollai et al., 1987; Mukaddam-Daher et al., 1994) warranted careful and immediate sample processing. Hence, blood samples were taken by jugular venepuncture and placed into prepared blood tubes containing potassium-EDTA (1.6 mg EDTA/ml blood, Sarstedt) and aprotinin (lyophilised aprotinin (500 KIU/ml blood, Sigma-Aldrich). They were then immediately iced on ice and centrifuged for 5 min at 1570 g and 4 °C. Plasma was then harvested, transferred into cryovials (TTP Techno Plastic Products), and frozen at −80 °C within 15 min after sampling.

Laboratory analysis

Human ANP has previously been shown to be stable at −80 °C for at least 12 months (Zolty et al., 2008). All samples were therefore analysed within 12 months after collection. The ANP was extracted and measured using a commercially available radioimmunoassay (RIA) kit (RIA kit RK-005-06: ANP1–18,Phoenix Pharmaceuticals) and a gamma-counter (GAMMMAMatic 1, Kontrol Analytical, W + W Electronic AG), following the manufacturer’s instructions. The target sequence recognised by the assay has previously been shown to be 100% homologous in horses and humans (Richter et al., 1998).

Data analyses and statistics

Data analyses and statistics were performed using commercial computer software (GraphPad Prism, version 5.03, GraphPad Software, SigmaStat, version 3.5, SyStat Software). Because of the low sample size, non-parametric and/or weighted regression analyses were performed and non-parametric statistics were chosen. Continuous variables were summarised using medians and ranges. The CPANP of the three Groups were compared using a Kruskal–Wallis one-way analysis of variance with Dunn’s post hoc test for multiple comparisons. Backwards stepwise multiple linear regression analyses were performed including all horses in Groups A and B to assess the relationship between CPANP and weight-corrected echocardiographic indices of LA and LV size and function. Linear measurements and area measurements were analysed separately. Measurements of LA and LV dimensions were forced into the equation, whereas the indices of LA and LV systolic function were allowed to be eliminated by stepwise backwards regression model. The level of significance was set at P < 0.05.
<table>
<thead>
<tr>
<th>Group</th>
<th>Horse</th>
<th>Breed</th>
<th>Bodyweight (kg)</th>
<th>Diagnosis</th>
<th>Cp ANP (pg/mL)</th>
<th>HR (bpm)</th>
<th>LADmax (cm)</th>
<th>LADmax [500] (cm)</th>
<th>LAAmax (cm²)</th>
<th>LAAmax [500] (cm²)</th>
<th>LAsxAmax (cm²)</th>
<th>LAsxAmax [500] (cm²)</th>
<th>Active LA FAC (%)</th>
<th>Passive LA FAC (%)</th>
<th>Active-to-total LA AC (%)</th>
<th>LVIDd (cm)</th>
<th>LVIDd [500] (cm)</th>
<th>LVFS (%)</th>
<th>LVIAd (cm²)</th>
<th>LVIAd [500] (cm²)</th>
<th>LV FAC (%)</th>
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</table>

Group A: Horses with heart disease and normal LA and LV size and mechanical function.
Group B: Horses with heart disease associated with LA enlargement, LA mechanical dysfunction, and/or LV enlargement.

Indices of active and passive LA mechanical function cannot be measured in absence of sinus rhythm.

Severe LA dilation. LA dimensions could not be measured because the heart size exceeded the maximum imaging depth of 30 cm.
Results

The CpANP expressed as median (min–max) in Group A, B and C were 12.5 (6.3–19.8), 53.5 (36.0–70.7), and 13.4 (7.2–34.0) pg/mL, respectively (Fig. 1, Table 1). The CpANP in Group B was significantly higher compared to Groups A and C, respectively (P < 0.05). There was no statistically significant difference in CpANP between Group A and Group C.

The multiple regression analysis showed that CpANP could be predicted from LADmax [500] (P = 0.033), whereas the LVIDd [500] (P = 0.743) and LV FS (P = 0.056) did not significantly add to the ability of equation to predict CpANP. Similarly, the CpANP could be predicted from LAAmax [500] (P = 0.003), whereas the effect of LVIAd [500] (P = 0.074) was not significant. Active LA FAC and LV FAC did not significantly add to the ability of the equation to predict CpANP and were removed from the model during the stepwise procedure. The power of the performed tests was 0.961 for linear indices and 0.987 for area indices. The relations of LADmax [500] and LAAmax [500], respectively, to CpANP are shown in Fig. 2.

Discussion

The results of this study indicated that CpANP was increased in horses with cardiac disease associated with cardiac chamber enlargement and/or dysfunction and that CpANP was directly related to the degree of LA enlargement.

The study population consisted of a group of horses with cardiac disease and normal LA and LV dimensions and mechanical function by echocardiography (Group A) and a group of horses with cardiac disease associated with LA enlargement, LA dysfunction, and/or LV enlargement (Group B). In Group A, the CpANP was slightly lower than values reported by Gehlen et al. (2007) in Warmblood horses with valvular regurgitation and normal chamber dimensions (20.1 ± 5.6 pg/mL, mean ± SD). In the latter study, CpANP in this group was not significantly different from CpANP in healthy Warmblood horses (21.0 ± 5.4 pg/mL) (Gehlen et al., 2007). Similarly, in our study there was no difference in CpANP between Group A and Group C (control group). Furthermore, CpANP in our control group was similar to the concentrations reported by Gehlen et al. (2007) in healthy Warmblood horses and similar to those reported in healthy Finnhorse trotters (18 ± 1 pg/mL, mean ± SE) and healthy Standardbreds (15 ± 2 pg/mL, mean ± SE) (Kokkonen et al., 1995). It could therefore be inferred that horses with cardiac disease and normal chamber dimensions could not reliably be differentiated from healthy horses by use of CpANP.

Conversely, CpANP may be suitable to identify left-sided chamber enlargement or dysfunction in horses with cardiac disease, since the CpANP in Group B was significantly higher compared to Group A, with no overlap between groups seen in this study. Notably, CpANP in Group B was markedly higher than the CpANP reported by Gehlen et al. (2007) in horses with enlarged LA but normal LV dimensions (22.9 ± 11.0 pg/mL), and in horses with both LA and LV enlargement (27.6 ± 17.4 pg/mL), neither of which was significantly different from healthy Warmblood horses (21.0 ± 5.4 pg/mL) in the aforementioned study.

The linear regression model revealed that CpANP in horses with cardiac disease was significantly related to LA dimensions, but not to LV dimensions or indices of LA and LV systolic function. The absence of a significant influence of LV dimensions in our study population was interesting, as enhanced ANP expression has been demonstrated in ventricular disease in experimental models and in human patients (Ruskoaho, 2003). However, this study only included a small number of horses and might not have allowed identifying all influencing factors. Furthermore, the influence of LA
dysfunction could not be assessed thoroughly as there was only one horse with LA dysfunction because of lone AF. Therefore, larger studies including horses with a wide spectrum of disease severity will be necessary to confirm these findings and to conclusively determine the relative influence of LA and LV enlargement and mechanical dysfunction as well as right heart enlargement and dysfunction on CpANP.

In the current study, the relation of CpANP to the two-dimensional area measurements seemed to be slightly stronger than the relation to the linear measurements. This might be explained by the fact that area measurements better represent LA dimensions, compared to linear measurements (Lang et al., 2006; Schwarzwald et al., 2007). This supported our contention that area measurements should be preferred or at least used in addition to linear measurements for echocardiographic quantification of chamber dimensions and chamber function in studies on cardiac biomarkers.

The absence of a well defined control group consisting of healthy horses without cardiac disease, as confirmed by echocardiography, was one of the limitations of this study. Furthermore, only a relatively small number of horses with a variety of cardiac disorders were enrolled. Therefore, the results did not permit a comprehensive assessment of the diagnostic value of CpANP in specific cardiac diseases, nor did they clearly differentiate the effects of atrial vs. ventricular enlargement on CpANP.

Conclusions

The results of this study indicated that measurement of CpANP in horses provides potentially useful data on cardiac chamber dimensions and that CpANP might be related to the severity of disease in horses with cardiac disorders. Larger prospective clinical studies are required to determine the diagnostic and prognostic value of ANP in horses with cardiac disease.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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References


