Dual natriuretic peptide response to volume load in the fetal circulation

T. Walther\textsuperscript{a,}\textsuperscript{*}, H. Stepan\textsuperscript{b}, R. Faber\textsuperscript{b}

\textsuperscript{a}Department of Cardiology and Pneumology, University Hospital Benjamin Franklin, Free University of Berlin, Hindenburgdamm 30, D-12200 Berlin, Germany
\textsuperscript{b}Department of Obstetrics and Gynaecology, University of Leipzig, Leipzig, Germany

Received 21 August 2000; accepted 7 November 2000

Abstract

Objective: To measure atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) in control fetuses and fetuses with Rhesus isoimmunisation before and after intravascular transfusion. The current study was designed to investigate the response of ANP and BNP to cardiac short-term and long-term volume load in the human fetus. Methods: Fetal blood samples were collected from 18 human fetuses (nine controls, nine anemic fetuses with Rhesus isoimmunisation before and after intravascular transfusion). Fetal ANP and BNP concentrations were measured and compared to maternal plasma levels. Results: Both ANP and BNP were significantly higher in fetal blood compared to the mothers. Fetuses with Rhesus isoimmunisation, characterized by long-term cardiac overload, showed significantly elevated ANP but not BNP concentration compared to the fetal controls (ANP: 80.8\textpm{}16.6 vs. 31.6\textpm{}7.7 pg/ml, \(P<0.05\)). However, short-term volume load due to intravascular transfusion leads to a significant increase in the fetal BNP- but not ANP-plasma level (BNP: 112.9\textpm{}14.1 vs. 64.8\textpm{}6.6 pg/ml, \(P<0.05\)). Conclusion: ANP and BNP respond differently to cardiac short- and long-term volume load in the fetal circulation. Therefore, the data suggest that in the fetus, similar to adults, ANP and BNP constitute a dual natriuretic peptide system responsive to changes in cardiac filling pressure. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Hemodynamics; Natriuretic peptide

1. Introduction

The role of endogenous vasoactive peptides as regulators of cardiac function in health and disease is an emerging area of fetal cardiovascular research. Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are secreted in an endocrine fashion mainly from the heart. Edwards et al. [1] could show, at least for ANP, that atrial stretch but not pressure is the principal determinant controlling the acute release of the peptide. Both peptides serve to maintain natriuresis, inhibit aldosterone secretion [2], and reduce intravascular volume and pressure, the combined actions controlling arterial pressure and cardiac filling pressure and output [3]. ANP and BNP act via a specific receptor located within the kidney, adrenal cortex, and the vascular system [4]. This receptor, the natriuretic peptide receptor A (GC-A), is guanylyl cyclase-coupled and located on the cell membrane. ANP in fetal circulation has been reported previously, but data on ANP level in fetal disease and the response to volume load are contradictory [5–7]. In contrast, nothing has been reported about BNP during fetal development. C-type natriuretic peptide as the third member of the natriuretic peptide family is detectable in fetal circulation but not influenced by fetal diseases [8].

Rhesus (Rh) isoimmunisation is characterised by a hyperdynamic circulation with increased blood flow velocities in arterial and venous vessels. Moreover, fetal anemia is compensated by an increased cardiac load and stroke volume [9,10]. Therefore, it was the aim of this study to investigate the ANP and BNP levels under the condition of long-term cardiac volume load compared to short-term volume load induced by intravascular transfusion.

\textsuperscript{*}Corresponding author. Tel.: +49-30-8445-4258; fax: +49-30-8445-4648.
E-mail address: walther@ukbf.fu-berlin.de (T. Walther).

0000-6363/01/$ – see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0000-6363(00)00303-5

Time for primary review 33 days.
2. Methods

Fetal blood was collected by cordocentesis from nine anemic fetuses with Rh isoimmunization without hydrops (mean gestational age: 30 weeks, mean hematocrit: <0.30). The site and direction of the umbilical cord at its placental insertion was defined by real-time ultrasonographic scanning and a 20-gauge needle was inserted in the umbilical vein. Fetal anesthesia was not performed. A 1-ml sample of fetal blood was immediately aspirated for the measurement of ANP and BNP before intravascular transfusion. Subsequently, intravascular transfusion was performed by packed red blood cells compatible with the mother to yield a donor hematocrit of 70%. Blood was infused into the fetus over 15–20 min to produce a closing fetal hematocrit of over 40%. The transfused blood volume was in the range of 20–30 ml. Immediately after transfusion a second fetal blood sample was taken to measure ANP and BNP after transfusion.

Blood samples were also obtained from nine fetuses undergoing cordocentesis with suspected but unconfirmed infection (mean gestational age: 21 weeks) which were used as a reference group. In addition, blood of all pregnant women was collected. Informed consent of all patients was obtained. Approval of the study was obtained from the medical scientific and ethical committees of the University of Leipzig.

All blood samples were drawn into tubes containing ethylene diamine tetra-acetic acid (EDTA). Immediately after sampling, plasma was separated by centrifugation at 4000×g for 10 min and frozen at −80°C. To measure ANP (200 μl) and BNP (200 μl) in fetal and maternal plasma highly sensitive radio-immunoassays without cross-reactivity for other natriuretic peptides or metabolites were used (Immundiagnostik, Bensheim, Germany).

Results are expressed as mean±S.D. Statistical comparisons were made by one-way analysis of variance and Tukey HSD test. Statistical significance was considered as P<0.05.

3. Results

The fetal ANP-plasma concentration of the control group was 31.6±7.7 pg/ml. Maternal plasma ANP was significantly lower (17.3±2.4 pg/ml, P<0.05, feto-maternal ratio: 1.82:1), whereas fetuses with Rh isoimmunisation showed a significantly increased ANP level (80.8±16.6 pg/ml, P<0.05). The volume load during intravascular transfusion did not alter the ANP plasma concentration (108.0±24.2 pg/ml; Table 1).

Also the BNP-plasma concentration of the fetal control group was 6.6±8.0 pg/ml. Maternal plasma BNP was load. Fetuses with Rh isoimmunisation show significantly increased cardiac output as a compensatory mechanism for the increased cardiac output as a compensatory mechanism for chronic congestive heart failure (CHF), plasma levels of ANP are significantly increased in the circulation secondary to enhanced atrial and ventricular synthesis and decreased ANP clearance in liver and kidneys [11]. Moreover, the secretion patterns of ANP and BNP vary with underlying cardiac disorders of CHF showing different degrees of overload in atria and ventricles [12]. While CHF can be taken as a model for a permanent cardiac overload in adults, fetuses with Rh isoimmunisation are characterised by long-term overload of the heart due to the increased cardiac output as a compensatory mechanism for fetuses with Rh anemia [9].

Our data show that already the fetal natriuretic peptide system reacts to short-term and long-term cardiac overload. Fetuses with Rh isoimmunisation show significantly higher ANP levels, whereas BNP was not influenced. In contrast, a short-term volume load by intravascular transfusion leads only to a significant rise in BNP. The finding that the ANP-plasma concentrations in fetuses after intravascular transfusion tend to rise only slightly is in contrast to Lang et al. [13] who found a significant increase of ANP after acute saline infusion in healthy volunteers. This contradiction may result from the exhausted ANP storage in the fetal heart in the state of chronic overload. Our data are also in line with the experimental work of Su et al. [14] who demonstrated that (64.8±6.6 pg/ml). The volume load during intravascular transfusion significantly elevated the BNP plasma concentration up to 112.9±14.1 pg/ml (P<0.05; Table 1). Maternal ANP and BNP plasma levels did not differ between pregnant women with or without Rh isoimmunisation (maternal controls vs. maternal Rh isoimmunisation for ANP: 17.3±2.4 vs. 16.8±1.9 pg/ml; for BNP: 26.8±3.9 vs. 28.1±3.4 pg/ml).

4. Discussion

During the last decade the components of the natriuretic peptide system have been intensively investigated in the normal heart and different kinds of cardiac diseases. In chronic congestive heart failure (CHF), plasma levels of ANP are significantly increased in the circulation secondary to enhanced atrial and ventricular synthesis and decreased ANP clearance in liver and kidneys [11]. Moreover, the secretion patterns of ANP and BNP vary with underlying cardiac disorders of CHF showing different degrees of overload in atria and ventricles [12]. While CHF can be taken as a model for a permanent cardiac overload in adults, fetuses with Rh isoimmunisation are characterised by long-term overload of the heart due to the increased cardiac output as a compensatory mechanism for fetuses with Rh anemia [9].

Our data show that already the fetal natriuretic peptide system reacts to short-term and long-term cardiac overload. Fetuses with Rh isoimmunisation show significantly higher ANP levels, whereas BNP was not influenced. In contrast, a short-term volume load by intravascular transfusion leads only to a significant rise in BNP. The finding that the ANP-plasma concentrations in fetuses after intravascular transfusion tend to rise only slightly is in contrast to Lang et al. [13] who found a significant increase of ANP after acute saline infusion in healthy volunteers. This contradiction may result from the exhausted ANP storage in the fetal heart in the state of chronic overload. Our data are also in line with the experimental work of Su et al. [14] who demonstrated that

<table>
<thead>
<tr>
<th></th>
<th>ANP (pg/ml)</th>
<th>BNP (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal (n=9)</td>
<td>17.3±2.4</td>
<td>26.8±3.9</td>
</tr>
<tr>
<td>Fetal controls (n=9)</td>
<td>31.6±7.7§</td>
<td>56.6±11.9§</td>
</tr>
<tr>
<td>Rh before transfusion (n=9)</td>
<td>80.8±16.6*</td>
<td>64.8±6.6</td>
</tr>
<tr>
<td>Rh after transfusion (n=9)</td>
<td>100.8±24.2</td>
<td>112.9±14.1#</td>
</tr>
</tbody>
</table>

* ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide. Values are mean±S.E.M.
§ P<0.05 fetal controls versus maternal.
* P<0.05 fetal controls versus before transfusion.
# P<0.05 before versus after transfusion.
a chronic cardiac overload in adult rats leads to a sevenfold increase of ANP mRNA after 24 h, whereas the BNP expression remains unaffected.

The immediate response of BNP but not ANP may result from an exclusive rapid BNP-gene activation. Magga et al. [15] have shown that an increase in atrial pressure leads to a fast increase of BNP-mRNA levels by enhanced transcriptional activation, whereas ANP-mRNA levels remain unchanged. In addition, our data agrees with results from Nakagawa et al. [16] who demonstrated that during cardiac hypertrophy BNP increases before ANP with a distinct regulation at transcriptional and post-transcriptional levels. We conclude, that the assumed function of BNP as an ‘emergency’ cardiac hormone against overload might be relevant already early in the fetus.

A number of studies show that the natriuretic peptide system matures early in the fetus. In mice, ANP-mRNA is detectable in myocardial cells at day 8 of embryogenesis [17]. The cardiac localisation of ANP changes during development from ventricular to predominantly atrial cardiomyocytes. Furthermore, rat fetuses respond to increases in intracardiac pressure with elevated plasma ANP similarly to mature animals, which indicates a functional natriuretic peptide system during fetal life [18].

While there were no data on BNP concentrations during fetal life, the previous findings of ANP in fetal circulation and fetal disease are contradictory. For instance, Ville et al. [7] reported increased ANP levels in anemic, acidicemic and hydropic fetuses. In contrast to Kingdom et al. [6] who described increased ANP levels after intravascular transfusion, Fisk et al. [5] reported increased ANP levels in non-hydropic and decreased ANP levels in hydropic fetuses after transfusion.

During the last decade many groups have provided new concepts about the natriuretic peptide system and its regulation in the adult heart and in cardiopathy. We demonstrate for the first time data about the regulation of this system in the fetus. In adults, Starling’s law maintains the balance between the venous return and the cardiac output by increasing stroke volume over a wide range [19]. Since the fetal heart with an immensely higher myocardial stiffness appears to operate along a very narrow range near the top of the ascending limb of the Starling curve, the fetus has a reduced ability to respond to cardiovascular stress with this mechanism [20]. Thus, the higher natriuretic peptide concentrations in fetal circulation compared to adults may indicate that the natriuretic peptide system is very important in controlling cardiac filling and volume in the fetus.

Obviously, ANP and BNP respond differently to cardiac short- and long-term volume load in fetal circulation. Thus, we conclude that in the fetus, similar to adults, ANP and BNP constitute a dual natriuretic peptide system responsive to changes in cardiac filling pressure.

References