Role of Natriuretic Peptides in cGMP Production in Fetal Cardiac Bypass

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Background. We previously showed cyclic guanosine 3′,5′-monophosphate (cGMP) levels increase with fetal cardiac bypass despite derangements in the placental nitric oxide pathway. The natriuretic peptides, atrial (ANP), brain (BNP), and c-type (CNP), are common indicators of cardiac distress, and an alternative pathway for cGMP generation. We hypothesized that these natriuretic peptides may account for the paradoxic rise in cGMP seen with fetal bypass.

Methods. Six ovine fetuses, 106 to 118 days' gestation, underwent cardiac bypass for 30 minutes and were followed for 120 minutes after bypass. Fetal plasma samples were collected before bypass, during bypass, and 30 and 120 minutes after bypass for natriuretic peptide analysis. Results were compared with 6 sham bypass fetuses and cGMP values from another 14 bypass fetuses (to avoid confounding effects of excess blood sampling). Fetal hemodynamics and metabolics were correlated to ANP, BNP, and CNP values. Statistical analysis was by analysis of variance, Student's t test, and best-fit correlations, with significance set at $p = 0.05$ or less.

Results. The ANP, BNP, and CNP increased with fetal bypass (674 ± 133 pg/mL, 151 ± 52 pg/mL, and 295 ± 45 pg/mL, respectively), remaining elevated after bypass, whereas sham concentrations remained stable at pre-bypass levels. Changes in ANP, BNP, and CNP positively correlated with rising cGMP. There was positive correlation between ANP and CNP and rising fetal lactate levels, but not to other physiologic parameters associated with placental dysfunction.

Conclusions. There is a substantial rise in natriuretic peptides seen with fetal bypass, likely in part a reflection of myocardial dysfunction. Further, the natriuretic peptide pathway may account for the paradoxic rise in cGMP seen with fetal bypass.

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The placental dysfunction reported after fetal cardiac surgery is of unknown etiology [1–6] but has led to immense interest in vasoactive substances and associated pathways whose disruption might be an underlying cause of elevated placental vascular resistance. Disruption of nitric oxide (NO) production, which stimulates cyclic guanosine 3′,5′-monophospate (cGMP) in vascular smooth muscle leading to vasodilatation, has been implicated in the placental dysfunction of fetal bypass. The same pathway has been implicated in pulmonary dysfunction in neonatal bypass models [3, 7]. Natriuretic peptides, however, also stimulate cGMP synthesis through a similar, but alternative pathway [8].

When catalyzed with guanosine-5′-triphosphate, cGMP is directly synthesized by two different types of guanylate cyclase enzymes: soluble guanylate cyclase and particulate guanylate cyclase. As seen in Figure 1, endothelial NO synthase (eNOS), a source of NO in the placenta, forms NO through direct stimulation of soluble guanylate cyclase production [9]. Particulate guanylate cyclase, however, is activated when atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) bind to their specific natriuretic peptide membrane receptors [10]. The ANP and BNP are found mainly in the atria and ventricles, respectively [11, 12], and bind to particulate guanylate cyclase-A receptors [10]. The CNP is produced primarily from endothelial cells [13, 14], in a wide variety of tissues such as brain, reproductive, and skeletal tissues, and binds preferentially to the particulate guanylate cyclase-B receptor [10, 15]. Recent studies have suggested an important role for CNP in local endothelial-mediated vasoregulation of the adult heart [16, 17], and also the fetal lung vasculature [15]. Increased levels of ANP, BNP, and CNP have also been shown to correlate with the severity of cardiac distress in congestive heart failure [17–19], and in response to cardiopulmonary bypass in children [20–23].

Our group has previously shown that in vivo NO levels acutely rise with the onset of fetal bypass, only to decline progressively after bypass [3]. In contrast, cGMP concentrations increase during bypass and paradoxically continue to rise after bypass when placental vascular resistance is elevated [3]. Therefore, it is unclear whether NO or some other factor is responsible for the observed rise

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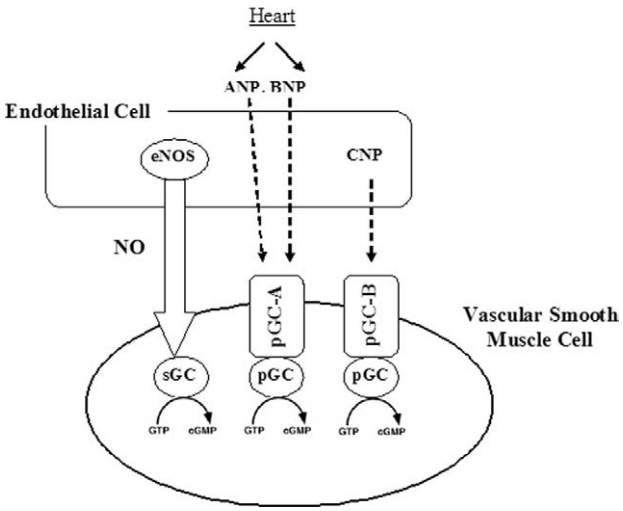

Fig 1. This diagram depicts the cyclic guanosine 3′,5′-monophosphate (cGMP) synthesis pathway through nitric oxide (NO) and natriuretic peptides. Atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) activate particulate guanylyl cyclase (pGC) through their respective receptors (pGC-A and pGC-B), and when catalyzed with guanosine triphosphate (GTP), form cGMP, leading to vasodilation. Nitric oxide derived from endothelial nitric oxide synthase (eNOS) activates soluble guanylyl cyclase (sGC), which also stimulates the synthesis of guanosine monophosphate (GMP), leading to vasodilation.

in cGMP concentrations. Thus, we became interested in the potential role of natriuretic peptides during fetal bypass as an alternate source of cGMP synthesis, particularly after bypass. This study tests our hypothesis that natriuretic peptides are upregulated with fetal bypass, thereby contributing to the increase of cGMP. We test this hypothesis by measuring fetal plasma levels of ANP, BNP, and CNP during and after bypass and correlating them with cGMP levels. Natriuretic peptide levels are also correlated with fetal metabolic and hemodynamic values to investigate possible relationships to changes in placental vascular resistance after fetal bypass.

Material and Methods

Surgical Instrumentation

Time-dated singleton and twin pregnant ewes (n = 6 experimental; n = 6 controls), 106 to 118 days of gestation (111 ± 3 days of gestation, 1.9 ± 0.5 kg body weight) were used in this study. Using methods we have previously described, ewes were fasted 24 hours before sedation with ketamine and valium, intubated endotracheally, and placed on 2% isoflurane and oxygen for anesthesia [2, 3, 24]. Catheters were placed in the ewes' femoral artery for blood gas measurement and femoral vein for intravenous fluid delivery. After midline laparotomy and a small hysterotomy, catheters were placed in the fetal femoral artery and vein for blood gas measurements and blood sampling, respectively. Through the same hysterotomy, an umbilical flow probe (Transonic Systems, Ithaca, NY) was placed to measure placental blood flow. Hemodynamic values were continuously recorded using a PowerLab data acquisition system (AD Instruments, Colorado Springs, CO), as described previously [2, 3, 24]. To address any potential unfound effects of sternotomy on natriuretic peptide levels, the experimental protocol was performed with a sternotomy in some control animals (n = 2) and experimental animals (n = 4). For the conduct of bypass, all fetuses were cannulated in the right jugular vein and the right carotid artery, as described previously [2, 3, 24]. The control group underwent all surgical procedures and cannulation, but the animals were not placed on bypass. All procedures were performed in accordance with Institutional Animal Care and Use Committee procedures in an Association for Assessment and Accreditation of Laboratory Animal Care–approved facility and complied with the "Guide for the Care and Use

Table 1. Fetal Blood Gases and Placental Hemodynamics Before, During, and After Bypass, and In-Group Analysis of Variance (ANOVA) With Least Significant Difference Post-Hoc Analysis

	Before Bypass	On Bypass	30 Minutes After Bypass	120 Minutes After Bypass	In-Group ANOVA
pH	7.28 ± 0.04	7.26 ± 0.07	7.25 ± 0.06	7.16 ± 0.12	**$p = 0.028$**
		$p = 0.682$	$p = 0.579$	**$p = 0.007$**	
PCO_2 (mm Hg)	62.3 ± 12.7	65.5 ± 10.6	69.0 ± 9.7	82.7 ± 13.7	**$p = 0.018$**
		$p = 0.624$	$p = 0.304$	**$p = 0.004$**	
PO_2 (mm Hg)	22.4 ± 3.4	24.7 ± 5.2	21.1 ± 5.6	17.4 ± 5.4	**$p = 0.049$**
		$p = 0.381$	$p = 0.535$	$p = 0.079$	
Lactate (mmol/L)	1.9 ± 0.4	2.5 ± 0.9	2.9 ± 1.5	4.4 ± 2.9	$p = 0.062$
		$p = 0.486$	$p = 0.275$	**$p = 0.010$**	
Umb Q (mL/min)	218 ± 85	339 ± 104	262 ± 96	137 ± 67	**$p = 0.028$**
		$p = 0.018$	$p = 0.369$	$p = 0.102$	
PVR (mm Hg · mL^{-1} · min^{-1})	0.188 ± 0.06	0.181 ± 0.07	0.178 ± 0.07	0.300 ± 0.19	$p = 0.169$
		$p = 0.908$	$p = 0.878$	$p = 0.082$	

Significance at $p = 0.05$ or less shown in bold.

PVR = placental vascular resistance; Umb Q = umbilical blood flow.