REVIEW

C-type natriuretic peptide in reproduction, pregnancy and fetal development

T Walther and H Stepan¹

Department of Cardiology and Pneumology, Free University of Berlin, Germany

¹Department of Obstetrics and Gynecology, University of Leipzig, Germany

(Requests for offprints should be addressed to H Stepan, Universitätsfrauenklinik, Philipp-Rosenthalstr 55, 04103 Leipzig, Germany;

Email: steh@medizin.uni-leipzig.de)

Abstract

C-type natriuretic peptide (CNP) belongs to the natriuretic peptide family that consists of three structurally related peptides with a 17-amino acid ring linked by a disulfide bond. In contrast to atrial and brain natriuretic peptides that are mainly cardiovascular hormones, CNP acts predominantly in an autocrine/paracrine fashion, is commonly considered to be an endothelial hormone with antimitogenic properties, and is characterized as a regulator of endochondral ossification. Its biological effects are mediated by an intracellular cGMP accumulation via specific membrane-bound guanylyl cyclase B (GC-B) activation. There is growing evidence that this peptide is also involved in various reproductive processes as well as in embryonic and fetal development. In rodents, CNP and its receptor are highly expressed in the uterus and ovaries with specific regulation during the estrous cycle. During pregnancy, CNP mRNA is detectable in mice embryos and shows an organ-specific expression in maternal reproductive tissues with the highest concentration in the placenta. This could indicate a defined biological function

of the CNP/GC-B/cGMP axis in gestation e.g. antagonizing vasoconstrictive peptides like angiotensin II. In humans, besides a postulated fetal *de novo* synthesis of CNP, both the peptide and its receptor are expressed in the placenta and myometrium with opposite regulation of CNP in pregnancies complicated by pre-eclampsia or intrauterine growth retardation. Since the maternal plasma levels do not reflect these alterations, one can conclude that this part of the natriuretic peptide system acts locally suggesting that CNP-stimulated cGMP release exhibits organ-specific effects.

Importantly, CNP has also become a peptide with a distinct role in male reproductive processes, since endocrine function of the testis and the regulation of penile erection are regulated by the CNP/GC-B axis. This review gives a comprehensive overview of the multiple functions of CNP in reproduction and pregnancy as well as in embryonic and fetal development.

Journal of Endocrinology (2004) **180**, 17–22

Introduction

The natriuretic peptide family consists of three structurally related peptides: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) (Levin *et al.* 1998). These peptides can influence a variety of homeostatic processes by the intracellular accumulation of cyclic GMP (cGMP) through two different membrane-bound guanylyl cyclase (GC)-coupled receptors (GC-A and GC-B) (Koller & Goeddel 1992). ANP and BNP are cardiac hormones that are produced predominantly by the atrium and ventricle respectively, and are thought to play an important role in the regulation of cardiovascular homeostasis (Maack 1996, Ganguly *et al.* 1999, Stein & Levin 1998). In contrast, CNP occurs in a

wide variety of tissues (Komatsu *et al.* 1991, Heublein *et al.* 1992, Totsune *et al.* 1994, Stepan *et al.* 2000a), where it may act locally as an autocrine/paracrine regulator through GC-B. Moreover, CNP is regarded as the 'endothelial component' of the natriuretic system and a local positive regulator of endochondral ossification (Chen & Burnett 1998, Chusho *et al.* 2001).

Two pathways have been described for the clearance of circulating natriuretic peptides: binding and internalization via the natriuretic peptide clearance receptor (Maack *et al.* 1987) and enzymatic degradation by neutral endopeptidase (EC 3.4.24.11), a widespread zinc metallo-peptidase that cleaves several peptide hormones by hydrolyzing internal peptide bonds at the amino side of hydrophobic residues (Kenny *et al.* 1993).

Figure 1 Schematic organization of the natriuretic peptide system (binding and degradation) with the three natriuretic peptides, two functional receptors and a clearance receptor without its own intracellular signaling. The scheme also shows the dual structure consisting of ANP-BNP/GC-A/cGMP and CNP/GC-B/cGMP.

A number of studies have shown that the natriuretic peptide system (NPS) can be divided into two axes with ANP and BNP (via GC-A) on one side and CNP (via GC-B) on the other (Fig. 1). Moreover, the ANP/BNP axis is not equally regulated but shows a functional dualism exhibiting different responses to various stimuli such as cardiac filling pressure (Yoshimura *et al.* 1993, Walther *et al.* 2001). Besides the cardiovascular as well as the endothelial functions of CNP, there are numerous hints of a biologically relevant role of this peptide in reproduction and fetal development: (1) in adult mice, the uterus and ovaries show the highest CNP expression (Stepan *et al.* 2000a); (2) estradiol induces CNP gene expression at least in the mouse uterus (Acuff *et al.* 1997); (3) CNP and its receptor are expressed in rat placenta (Cameron *et al.* 1996) and are modulated in rat ovary and uterus by the estrous cycle, with maximal expression at proestrus (Dos Reis *et al.* 1995, Huang *et al.* 1996); (4) for human reproductive tissues, it can be demonstrated that CNP expression is altered in pathological states of pregnancy such as intrauterine growth retardation (IUGR) (Stepan

et al. 2002a); and (5) in the male reproductive system, CNP is involved in the regulation of testicular and erectile function (Middendorff *et al.* 2000, Kthe *et al.* 2003). Therefore, the aim of this review is to provide a comprehensive overview of the multiple functions of CNP in reproduction and pregnancy as well as in embryonic and fetal development.

CNP in female reproductive tissues

Following the first description of CNP in the porcine brain (Sudoh *et al.* 1990) and its characterization as a neurotransmitter and cardiovascular peptide (Clavell *et al.* 1993), the first hints regarding a possible function of CNP in reproduction were described. In non-pregnant mice, the uterus and ovaries are the organs with the highest CNP mRNA concentrations, exceeding the CNP levels of organs such as the brain or kidney (Stepan *et al.* 2000a). Detailed investigations showed that the uterine CNP expression is obviously controlled by other

hormone systems. For instance, an intraperitoneal infusion of estradiol increases uterine CNP in a dose-dependent fashion in ovariectomized mice (Acuff *et al.* 1997). In rats, uterine CNP content is modulated by the estrous cycle with the highest expression at proestrus (Huang *et al.* 1996). This cycle dependency can result from uterine mass and fluid content changes or from estradiol variations (Acuff *et al.* 1997). The regulation of CNP in reproductive tissues from non-pregnant animals occurs in parallel with a high expression of the CNP receptor (GC-B), and is, for example, a hundred times higher in the uterus compared with the GC-A receptor (Dos Reis *et al.* 1995). Also in the rat ovary, both CNP and GC-B show a time-dependent expression over the estrous cycle (Jankowski *et al.* 1997). As far as the role of the CNP/GC-B axis in the ovary is concerned, a modulation of follicular atresia through CNP has been postulated. McGee *et al.* (1997) showed that 8-bromo-cGMP, an inhibitor of the guanylyl cyclase signaling, was able to decrease follicular atresia, which appears mainly in follicles at the preantral to antral transition. Beside the regulated expression of CNP and its receptor, these findings point indirectly to a physiological role of the NPS, particularly CNP, in female reproductive organs.

CNP in male reproductive tissues

The further characterization of CNP has provided more and more evidence that this hormone is also involved in male reproductive processes. Both CNP and its receptor are expressed in human testis (Middendorff *et al.* 1997, 2000). It was demonstrated that the factor in porcine seminal plasma that causes an increase in cGMP was indeed CNP (Chrisman *et al.* 1993). The third member of the NPS also influences testicular endocrine function such as testosterone release via cGMP, and is also involved in the regulation of Leydig and Sertoli cell function (Middendorff *et al.* 1997, 2000). Possible paracrine actions include relaxation of seminiferous tubules in order to regulate sperm transport and testicular blood supply respectively (Middendorff *et al.* 1997). Thus, the CNP/GC-B/cGMP cascade leads to a variety of biological effects in the testis such as modulating spermatozoa motility, testicular germ cell development and testosterone synthesis (Middendorff *et al.* 2000).

Since sildenafil, an inhibitor of the cGMP-degrading phosphodiesterase type 5, has been introduced into broad clinical practice, it has become clear that cGMP-mediated processes not only regulate autocrine/paracrine testicular functions, but also have an impact on erectile function. This has been supported by the observation that in rabbits and rats CNP binds to the GC-B receptor of the cavernosal membrane and causes smooth muscle cell relaxation within the penis (Kim *et al.* 1998), and by the finding of Kthe *et al.* (2003) that the GC-B receptor is

expressed in the human corpus cavernosum, suggesting that CNP influences penile erection. Furthermore, there is an observed cross-talk between the CNP/GC-B axis and the receptors of cAMP elevating peptides such as vaso-active intestinal peptide and pituitary adenylate cyclase activating peptide in regulating erectile function (Guidone *et al.* 2002). In conclusion, CNP has an impact on the biological function of the male reproductive tract regarding endocrine testicular regulation and penile erection.

Maternal CNP regulation during pregnancy

Pregnancy induces a 38-fold increase in uterine cGMP production (Itoh *et al.* 1998), indicating activation, besides other cGMP-generating systems such as the kallikrein-kinin system, especially of the NPS. In mice, uterine CNP mRNA concentrations increase up to sevenfold during pregnancy, whereas in the ovaries these levels decrease to ten percent of those of non-pregnant controls (Stepan *et al.* 2001). These reciprocal and gestational age-dependent changes of CNP expression have led to hypotheses about the biological control and functions of CNP during pregnancy. First, the ability of CNP to relax smooth muscle cells may have a protective effect during gestation by inhibiting uterine contractions (Drewett *et al.* 1995). Interestingly, the natriuretic peptide-induced relaxation seems to be independent of cGMP, since blockage of guanylyl cyclase A or B activation did not diminish this effect in the myometrium (Carvajal *et al.* 2001). Shear stress, a CNP-inducing factor (Okahara *et al.* 1995), caused the observed CNP increase within the growing uterus. Moreover, since CNP is produced in the theca-interstitium, which is under luteinizing hormone (LH) control (Jankowski *et al.* 1997), the decrease in ovarian CNP expression with advancing gestational age may result from a lack of LH stimulation.

Using *in situ*-hybridization, Cameron *et al.* (1996) showed a strong placental CNP expression in mice starting at day 10.5 post coitus (pc), with the strongest expression localized around the large maternal blood vessels. Our group detected CNP mRNA in murine placentas from day 9.5 pc with a maximum at day 15.5 pc (Stepan *et al.* 2001). Thus, the course of placental CNP expression is similar to that of ANP, which peaks at day 16 in rats (Huang *et al.* 1992). The direct comparison of CNP expression between placenta, uterus, and ovaries showed that the placental CNP mRNA concentration exceeds that of the uterus and ovary. The concept of a functioning local NPS in the placenta is further supported by the finding that both GC-A and GC-B are expressed in the human placenta at term (Hatjis & Grogan 1988, Itoh *et al.* 1994), and their expression patterns change greatly during pregnancy. Whereas the clearance receptor is down-regulated, the soluble and particulate guanylyl cyclases

increase specifically in the ovine uterine but not systemic arteries (Itoh *et al.* 1998).

CNP mRNA was detected in human placenta and myometrium. In these tissues CNP expression levels did not differ between term and preterm pregnancies (Stepan *et al.* 2002a). Pregnancies with IUGR or pre-eclampsia show an opposite regulation of CNP with a decrease in the placenta and an increase in the myometrium compared with normal pregnancies; this could indicate a compensatory or causative organ-specific function of the peptide in human reproductive tissue under these pathophysiological conditions (Stepan *et al.* 2002a,b). Interestingly, both pregnancy disorders are a clinical consequence of placental insufficiency and are characterized by comparable CNP expression pattern in the placenta and myometrium. However, in contrast to CNP, BNP at the protein level is up-regulated in the placenta of pre-eclamptic patients (Walther *et al.* 2002) indicating different functions for the two NPS axes.

Interestingly, maternal CNP plasma levels were not influenced by the IUGR situation or in pregnancies with hypertensive disorders such as pregnancy-induced hypertension or pre-eclampsia (Stepan *et al.* 1998a, 1999, 2002a), emphasizing that in the above mentioned tissue CNP regulation does not reflect the circulating peptide concentrations. However, other factors can influence maternal peripheral CNP concentrations. For instance, CNP is significantly increased after volume load before cesarean section (Stepan *et al.* 1998b), which leads to the postulate that systemic CNP release can be triggered by rapid volume expansion, as is typical for ANP. Animal models support this concept of a systemic CNP response to volume (Borgeson *et al.* 1998). Secondly, CNP is significantly up-regulated in labor (Stepan *et al.* 1998b), which could be related to a known interaction between CNP and catecholamines that are increased during uterine contractions (Vatta *et al.* 1997).

Further studies will prove whether NT-proCNP(1–50), the biologically inactive fragment of the CNP precursor which is increased in human patients with congestive heart failure (Prickett *et al.* 2001), may better reflect the local changes due to its longer half-life.

CNP and fetal development

A number of studies have described early expression of CNP in embryonic and fetal tissues, indicating that this component of the NPS has functions during the first stages of intrauterine development. Using *in situ*-hybridization, CNP mRNA could be detected in distinct areas of the mouse brain at day 10.5 pc (in the ventricular zone of the pons adjacent to the floor of the fourth ventricle and in the cerebral cortex) and in the spinal cord (Cameron *et al.* 1996). Our group was able to detect CNP mRNA in both head and trunk of mouse embryos. For both parts of the embryo, high mRNA concentrations were found at day

9.5 pc with no significant difference between the head and trunk; this was followed by a significant decrease. A further peak in the heads of embryos was visible at day 18.5 pc, whereas CNP expression in embryos' trunks increases continuously until birth (Stepan *et al.* 2001). Although these alterations in CNP mRNA cannot be directly related to a physiological function during embryonic development, further studies investigating embryos deficient in, or overexpressing CNP will clarify this issue.

A recent study identified the human heart as a CNP-producing organ, at least in the pathophysiological condition of chronic heart failure (Kalra *et al.* 2003). Although CNP is not yet expressed in the heart of mouse and human embryos (Takahashi *et al.* 1992, Cameron *et al.* 1996), neonatal mice already show low cardiac CNP mRNA concentrations that increase up to eightfold in adult animals (Stepan *et al.* 2001). Thus, it seems unlikely that CNP is a physiologically relevant component of the cardiac NPS during fetal development and should be considered as an independent hormone within the NPS. Since Doi and co-workers could show that CNP expression is suppressed by the vascular endothelial growth factor (VEGF) (Doi *et al.* 1996) and is co-expressed with components of the VEGF system in embryoid bodies, an *in vitro* model of developing embryos (Doi *et al.* 1997), a role for CNP in vascular cell differentiation and vasculogenesis can be assumed. However, the antimitogenic and antiproliferative effect of CNP on cultured adult vascular cells (Furuya *et al.* 1991) has not been demonstrated in direct *in vitro* or *in vivo* assays using embryonic vascular cells or vascularization assays.

In human second trimester fetuses, CNP plasma concentrations could be measured after cordocentesis in normal fetuses, fetuses with rhesus isoimmunization before and after intravascular transfusion and fetuses with structural malformations (Stepan *et al.* 2000b). However, the source of CNP production in the human fetus remains to be elicited. In contrast to ANP and BNP (Walther *et al.* 2001), the fetal CNP plasma concentrations remain stable in the investigated fetal diseases and after volume load during intravascular transfusion. This could be expected because a long-term up-regulation of CNP would probably cause skeletal malformations as reported for mice with CNP overexpression (Y Ogawa, personal communication). Moreover, fetal CNP plasma levels are higher than previously measured maternal concentrations, suggesting that the fetus expresses CNP independently from the maternal circulation (Stepan *et al.* 2000b). This is supported indirectly by the finding that ANP does not cross the placenta (Deloof *et al.* 1995). This has not yet been investigated for CNP but because of the structural homology within the natriuretic peptides one can expect that CNP also does not cross the placenta. This assumption and the finding of higher fetal CNP plasma concentrations compared with the maternal circulation indicate that the developing fetus is able to synthesize CNP *de novo*.

Transgenic animal models

The generation of animal models with over-expression or disruption of genes has given further insight into the physiology of the NPS. The offspring of CNP-deficient mice show severe dwarfism due to impaired endochondral ossification. Neonatal mice are slightly growth retarded and show a high mortality, with 70% of the pups dying during the first 100 days (Chusho *et al.* 2001). However, surviving males and females are fertile. Up to now, a detailed histomorphological and endocrine analysis of reproductive tissues in these animals is still lacking. Transgenic animals over-expressing CNP also show bone malformations (Y Ogawa, personal communication). Interestingly, investigation of the *Nppc* $-/-$ mice that lack the clearance receptor and mice over-expressing BNP also reveals typical signs of achondroplasia (Suda *et al.* 1998, Matsukawa *et al.* 1999, Chusho *et al.* 2000). While the clearance receptor deficiency leads to the elevation of CNP concentrations comparable to that in CNP-over-expressing mice, Chusho *et al.* (2000) demonstrated by cross breeding of the BNP model with GC-A-deficient mice that these malformations are mediated by an 'unspecific' BNP overstimulation of the GC-B receptor.

In conclusion, one of the major biological functions of CNP in the fetus is the regulation of bone growth via chondrocyte proliferation and cartilage matrix production (Mericq *et al.* 2000).

Conclusions

The recent literature and our own investigations on the role of C-type natriuretic peptide in reproduction, pregnancy and fetal development indicate mRNA expression in the reproductive target organs, its regulation under pathophysiological conditions and preliminary data for a distinct function of CNP in defined situations. Although the primary signaling pathway with the CNP/GC-B/cGMP axis seems to be identified and is singular for this hormone, further investigations are needed to characterize intracellular signal cascades and CNP target genes to illustrate the importance of this third member of the NPS. However, the mechanisms by which CNP mediates its biological functions are multiple, and the inhibition of cell proliferation and differentiation and the vasorelaxant action serve only as two examples.

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Received 18 July 2003

Accepted 16 October 2003