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Research Report

# Nitric oxide synthase activity and expression are decreased in the paraventricular nucleus of pregnant rats

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ABSTRACT

Pregnancy is characterized by elevated heart rate and decreased total peripheral resistance and arterial blood pressure. Plasma volume is expanded and plasma osmolality is decreased, yet vasopressin secretion in pregnant animals, including humans, is no different than levels in the nonpregnant state. Although reflex compensatory sympathoexcitation is suppressed, baseline sympathetic nerve activity to the heart and vasculature is well maintained or slightly elevated in pregnancy. Clearly there are central nervous system (CNS) adaptations in systems for regulation of cardiovascular and body fluid homeostasis in pregnant animals. The paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus are important CNS sites for control of sympathetic nerve activity and vasopressin secretion. Nitric oxide (NO), an important neuromodulator in these hypothalamic nuclei, contributes to tonic inhibition of neurosecretory and pre-autonomic neurons. Alterations in NO within the PVN and SON could contribute to changes in regulation of vasopressin and sympathetic nerve activity in pregnancy. In the present study, nitric oxide synthase (NOS) activity (NADPH-diaphorase staining), neuronal NOS (nNOS) protein, and nNOS mRNA were assessed in nonpregnant estrus stage and near-term pregnant rats. nNOS mRNA, protein, and activity were greater in the PVN than in the SON. In the PVN only, pregnancy was associated with significant decreases in all three measurements for assessment of nNOS. Thus decreased NO production and relative disinhibition of the PVN may contribute to maintenance of baseline vasopressin secretion and baseline sympathetic nerve activity in the pregnant state.



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## 1. Introduction

Pregnancy is associated with increased heart rate, increased blood volume, decreased vascular sensitivity to endogenous vasoconstrictors and decreased total peripheral resistance and arterial blood pressure (DeSwiet, 1988; Lindheimer and Katz, 1992). Increased synthesis of nitric oxide (NO) by the vascular endothelium likely contributes to vasodilation and decreased vasoconstrictor responses in pregnant animals, including humans. However, peripheral NO mechanisms cannot fully account for changes in blood pressure regulation in pregnancy (Brooks et al., 2001; McLaughlin and Conrad, 1995; Sladek et al., 1997). In addition to changes in the peripheral vasculature, the central nervous system (CNS) homeostatic systems for control of both blood volume (Lindheimer and Davison, 1995) and the autonomic nervous system (Brooks et al., 1997; Greenwood et al., 2001; Masilamani and Heesch, 1997; O'Hagan and Casey, 1998) are reset and contribute to the hemodynamic profile characteristic of normal pregnancy. In addition to its peripheral actions, NO is an important neuromodulator in hypothalamic nuclei involved in control of blood volume and cardiovascular regulation (Badoer, 2001; Coote, 1995).

Nitric oxide is a highly diffusible gaseous signaling molecule that is synthesized on demand by the enzyme, nitric oxide synthase (NOS). Once formed, NO diffuses from nerve terminals and/or cell bodies to act as a nontraditional neurotransmitter/neuromodulator influencing cells in the vicinity of its release (Esplugues, 2002). Neuronal NOS (nNOS) is the predominant isozyme responsible for the formation of NO in the SON and PVN (Bhat et al., 1996; Gingerich and Krukoff, 2005). In both the SON and PVN, nNOS is expressed in magnocellular neurons that synthesize and release vasopressin and oxytocin. In addition, nNOS is expressed in autonomic related parvocellular neurons in the PVN that project to the rostral ventrolateral medulla (RVLM) and/or the intermediolateral cell column of the spinal cord and play an important role in control of sympathetic nerve activity and blood pressure (Badoer, 2001; Swanson and Sawchenko, 1983; Stern, 2004; Stern and Zhang, 2005). NO tonically inhibits neuronal activity in hypothalamic magnocellular and preautonomic neurons mainly due to pre-synaptic facilitation of GABAergic neurotransmission (Horn et al., 1994; Li et al., 2003; Stern, 2004). Regulation of nNOS is dependent on neuronal activity (Kadekaro, 2004) and changes in expression and activity of nNOS in the hypothalamus have been implicated in many physiological and pathophysiological adaptations in neurohumoral control of the circulation (DiCarlo et al., 2002; Krukoff, 1999; Mueller et al., 2006; Patel et al., 1996; Zhang et al., 1998b).

Although plasma volume is increased by approximately 40% and plasma osmolality is decreased by 10 mOsm during pregnancy, plasma vasopressin levels are no different than in the nonpregnant state. The osmotic threshold for vasopressin secretion is decreased, but sensitivity to osmotic stimuli and to changes in blood volume and arterial pressure is not changed by pregnancy (Lindheimer and Davison, 1995; Summy-Long and Kadekaro, 2001; Ward et al., 1991). Thus, resetting of the osmotic threshold for vasopressin secretion contributes to the maintenance of the expanded blood volume in pregnancy. Regulation of sympathetic nerve activity is also changed by pregnancy. While arterial baroreflex sympathoexcitation is blunted, baroreflex sympathoinhibition is slightly augmented in term pregnant rats (Masilamani and Heesch, 1997). Independent of the arterial baroreflex, baseline GABAergic inhibition of the RVLM is greater in pregnant compared to nonpregnant rats (Kvochina et al., 2007). Increased baseline inhibition at the level of the RVLM likely contributes to attenuated baroreflex sympathoexcitation in pregnant rats. However, increased GABAergic inhibition of the RVLM does not explain the fact that baseline sympathetic tone to the heart (Cohen et al., 1988; Lucini et al., 1999) and vasculature (Greenwood et al., 2001; Masilamani and Heesch, 1997) is slightly elevated in pregnant animals, including humans. In male rats, inhibition of neuronal activity in the PVN reduces arterial pressure and renal sympathetic nerve activity, suggesting that the PVN may contribute to baseline sympathetic tone (Dampney et al., 2000; Zhang and Patel, 1998a). Therefore it is possible that changes in sympathoexcitatory output from pre-autonomic neurons in the PVN could contribute to the maintenance of baseline sympathetic outflow in pregnancy and that adaptations in NO mechanisms may play a role.

There are conflicting reports in the literature regarding expression of nNOS and nNOS activity in term pregnancy. Some studies have provided evidence for increased nNOS in the hypothalamus of term pregnant animals (Popeski et al., 1999; Woodside and Amir 1996; Xu et al., 1996). In contrast, others have reported no change or decreased nNOS in the PVN and/or SON of term pregnant animals (Daubert et al., 2007; Okere and Higuchi, 1996; Srisawat et al., 2000). Differences in methods for assessing nNOS, stage of the cycle in the nonpregnant control group, and the day of pregnancy studied in these experiments could contribute to the conflicting results.

Experiments in the current study tested the hypothesis that in near-term pregnant rats, nNOS is decreased in the PVN and SON, which would be consistent with decreased tonic inhibition of these regions. nNOS activity (NADPH-diaphorase staining), nNOS protein, and mRNA for nNOS in the PVN and SON were evaluated in a controlled set of experiments in similarly treated near-term pregnant and nonpregnant female rats. Experiments in cycling nonpregnant rats were performed in the estrus stage of the cycle when ovarian hormones are consistent and low (Butcher et al., 1974; Haim et al., 2003). Experiments in near-term pregnant rats were performed on the morning of day 21 which is 1.5–2 days prior to expected delivery (Francis et al., 2002; Kobayashi et al., 1999) and is characterized by elevated estrogen and progesterone levels (Bridges, 1984; Concas et al., 1998; Garland et al., 1987; Kobayashi et al., 1999).

## 2. Results

### 2.1. *Effect of pregnancy*

Table 1 contains a summary of baseline information for the 37 rats used in these experiments. All nonpregnant rats were in the estrus stage of the estrous cycle. Near term pregnant rats weighed significantly more than nonpregnant rats. Number of