NOS inhibition restores renal responses to atrial distension during pregnancy

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Tam, Siu Lin, and Susan Kaufman. NOS inhibition restores renal responses to atrial distension during pregnancy. Am J Physiol Regulatory Integrative Comp Physiol 282: R1364–R1367, 2002.—Nitric oxide (NO) biosynthesis increases during pregnancy and has been shown to suppress baroreceptor activity. The renal response to a simulated increase in circulating blood volume (atrial distension) is also attenuated at this time. We hypothesized that blocking NO biosynthesis during pregnancy would restore the renal response. Female rats were implanted with indwelling intracardiac balloons and central venous cannulas. After recovery, they were mated, and on day 14 of pregnancy, osmotic minipumps containing the NO synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME) or its inactive enantiomer NG-nitro-D-arginine methyl ester (D-NAME) (120 mg/2 ml at 10 μg/min) were implanted. In response to atrial distension (1 h), urine output increased in the D- and L-NAME-treated virgin rats. During pregnancy (day 20), this response was attenuated in the D-NAME-treated, but not the L-NAME-treated, animals, i.e., after a simulated increase in circulating blood volume, inhibition of NO biosynthesis restored the renal response of pregnant rats to that seen in virgin animals. We conclude that, during normal pregnancy, increased NO biosynthesis blunts the reflex renal response to atrial distension.

atrial volume receptors; natriuresis; diuresis

CARDIOVASCULAR HOMEOSTASIS is significantly altered during pregnancy. There is a marked increase in blood volume (16), associated with increased cardiac output (21). There is also sodium retention, despite the increase in glomerular filtration rate (3). Atrial distension significantly increases renal output in virgin rats but not in term pregnant rats (15). The pregnant animal thus fails to recognize an increase in venous return to the heart. These findings suggest that there is a perceived underfilled state of the vascular compartment in pregnancy. This would thus contribute to the maintenance of blood volume expansion and fetal growth (23).

Although there is evidence that atrial volume receptor activity may be reduced during pregnancy (8, 10, 11), the mechanism underlying this alteration is still not known. The physiological adaptations to pregnancy (increased blood volume, increased cardiac output, and sodium retention) are accompanied by an increase in nitric oxide (NO) biosynthesis (2, 7, 25). Because NO has been shown to suppress baroreceptor activity (18), we proposed that NO might also be responsible for blunting atrial volume receptor activity. We had previously shown that chronic inhibition of NO biosynthesis reduces blood volume in pregnant, but not virgin, rats (26). This could be attributed, at least in part, to restoration of the reflex natriuresis/diuresis that normally follows an increase in circulating blood volume, a response that is attenuated during pregnancy (15). We hypothesized that, by blocking NO biosynthesis in pregnant rats, the renal response to atrial distension would be restored to that seen in virgin rats. Accordingly, urine output was measured in virgin and pregnant rats, which had been implanted with intracardiac balloons and pretreated with the NO synthase (NOS) inhibitor NG-nitro-L-arginine methyl ester (L-NAME) or its inactive enantiomer NG-nitro-D-arginine methyl ester (D-NAME). We chose to use the exact experimental conditions, chronic L-NAME administration, under which we had previously investigated the effect of NOS inhibition on blood volume (26).

MATERIAL AND METHODS

The experimental procedure in the present study was approved by the local Animal Welfare Committee in accordance with the guidelines issued by the Canada Council on Animal Care. All animals were killed with an anesthetic overdose of pentobarbital sodium at the completion of the studies.

Animals and housing. A total of 43 female rats (250–300 g) was used in this study. Long-Evans rats were obtained from Charles River Canada (St. Foy, Quebec, Canada) and housed in a temperature- and humidity-controlled animal facility with a 12:12-h light-dark cycle (light 0700–1900) for 1 wk before use. They were maintained on the LabDiet rat chow (PMI) throughout the entire experiment.

Surgery. Surgery was carried out in all animals under pentobarbital sodium anesthesia (62 mg/kg body wt ip) and sterile conditions. Silastic cannulas (0.51-mm ID, 0.94-mm OD; Dow Corning) were implanted nonocclusively into the inferior vena cava for saline infusion (13). Small inflatable balloon cannulas were passed down the right jugular vein and secured to the clavicle so that the tip of the balloon lay at the venoatrial junction (14). The anatomy of the rat is such...
that inflation of the balloon (50 μl) does not interfere with venous return to the heart. The urinary bladder was truncated to one-third of the original size to allow small and frequent quanta of urine excretion. After the initial surgery, the rats were allowed to recover to their preoperative weights, about 2 wk, before further experimental procedures were carried out.

**Experimental protocol.** The rats were randomly allocated to the following groups: 1) virgin rats with atrial distension, 2) virgin rats without atrial distension, 3) 21-day pregnant rats with atrial distension, and 4) 21-day pregnant rats without atrial distension. The rats in groups 3 and 4 were subjected to vaginal smears and placed with the male rats at proestrus. The success of pregnancy was estimated by the increase in body weight 7 days later. At day 14 of pregnancy, all animals, including the virgin rats, were implanted subcutaneously with osmotic minipumps (model 2ML1, DURECT) containing either D- or L-NAME (120 mg/2 ml at 10 μg/min; Calbiochem). Five days later (day 19), the rats were transferred to metabolic cages, for ease of accessing the cannulas, and kept there for 1-day acclimatization. On day 20, the rats were infused with saline (3 ml/h) via the inferior vena cava cannula for 3 h (2 h before and 1 h after inflating the intra-atrial balloon). Urine was collected and measured for 1 h before and 1 h during balloon inflation.

**Na output.** Micromolar concentration of urinary Na was measured using a Micro-Combination sodium electrode (model 9811, Orion Research).

**Statistics.** Throughout this paper, means ± SE are given. Student’s paired t-test was used to examine statistical significance of changes in renal output in response to atrial distension. Two-way analysis of variance and Student-Newman-Keuls method were applied to examine the presence of statistical significance and the loci of significance, respectively, among the baseline outputs. For all above statistical analyses, P < 0.05 was regarded as significant.

**RESULTS**

There were no significant differences in baseline urine output between the D- and L-NAME-treated virgin rats, between the D- and L-NAME-treated pregnant rats, or between the virgin and the pregnant rats (Fig. 1).

Atrial distension, induced by inflating the balloon located at the venoatrial junction, significantly increased urine output in both the D- and L-NAME-treated virgin rats (Fig. 1A). During pregnancy, the atrial distension-induced increase in urine output was abolished in the D-NAME-treated (control) rats (Fig. 1C). However, urine output was completely restored to prepregnant levels in the L-NAME-treated (NOS inhibition) term pregnant rats (Fig. 1C).

Before atrial distension, there were no significant differences in Na output between the D- and L-NAME-treated virgin rats or between the D- and L-NAME-treated pregnant rats (Fig. 2). However, mean Na output before atrial distension for the virgins was significantly higher than that for pregnant rats (Fig. 2, A and C).

In response to atrial distension, Na output was significantly increased in both D- and L-NAME-treated virgin rats (Fig. 2A). During pregnancy, this increase was completely abolished in the D-NAME-treated, but not in the L-NAME-treated, pregnant rats (Fig. 2C).

The groups in which D- or L-NAME-treated virgin or pregnant rats were not subjected to atrial distension were used as time controls for those subjected to atrial distension. No significant changes in urine or Na output over the 2-h experimental period were found in any of the time control groups (Fig. 1, B and D, and Fig. 2, B and D). Na output in the virgin rats was, however, significantly higher than that in the pregnant rats (Fig. 2, B and D).

**DISCUSSION**

In virgin rats, atrial distension caused an increase in urine volume and renal Na output. L-NAME did not alter this response (Figs. 1A and 2A). In control preg-
nant rats treated with D-NAME, there was no renal response to atrial distension (Figs. 1C and 2C). However, the response was restored by pretreating the animals with the NOS inhibitor L-NAME, i.e., endogenous NO interferes with the renal response to atrial distension during pregnancy.

It has been well established that blood volume increases during normal pregnancy (5, 16). We (26) and others (22) have demonstrated that long-term administration of L-NAME reverses these pregnancy-induced changes. However, L-NAME does not alter basal water intake or urine output in female (26) or male (19) rats. This suggests that L-NAME does not cause primary polydipsia or renal Na retention but rather alters homeostasis of blood volume.

Several studies have shown there to be a generalized decline in the sensitivity of autonomic neural reflexes during pregnancy. Studies by Heesch and Rogers (9), Masilamani and Heesch (17), and Brooks et al. (6) have shown that neural reflex responses to changes in blood pressure, mediated by the arterial baroreceptors, are attenuated during pregnancy. Reflex control of blood volume by the atrial volume receptors is similarly blunted during pregnancy (8, 12, 15, 20).

To study volume regulation during pregnancy, different methods of volume loading have been developed (1, 7). However, given the difficulty of providing equipotent hypervolemic stimuli to virgin and pregnant animals that differ greatly in body weight and extracellular volume, the findings of these studies must be interpreted with caution. This problem has been circumvented by Kaufman (14), by direct localized distension of the venoatrial junction with an indwelling balloon. This delivers an equipotent stimulus directly to the atrial volume receptors (15).

Using this technique, namely the indwelling intracardiac balloon, we have shown in the present study that atrial distension significantly increases urine and Na output in both D- and L-NAME-treated virgin rats, i.e., that the reflex response to atrial distension does not depend on NO and that the normal kidney is still capable of mounting an increase in urine volume and Na output despite inhibition of NO biosynthesis. We have also shown that, while pregnancy attenuates the atrial distension-induced increase in urine and Na output in D-NAME-treated pregnant rats, blocking NO production with L-NAME completely restores the response.

NOS inhibition increases mean arterial pressure in both virgin and pregnant rats (26). Given that there is interaction between the arterial baroreceptors and the atrial volume receptors (24), this could potentially influence the renal response to atrial distension. However, because L-NAME did not alter the magnitude of the renal response to atrial distension in the virgin animals, and because baseline blood pressure is very similar in pregnant and virgin rats (26), it is unlikely that the increase in blood pressure associated with administration of L-NAME could be held responsible for restoring the renal response during pregnancy.

**Perspectives**

It has been shown that NO exerts both glomerular and tubular effects in the kidney, resulting in increased Na excretion (4). NOS inhibition might thus be expected to reduce renal output. However, it had no effect on basal output in either virgin or pregnant animals and increased stimulated output in the pregnant animals. Moreover, despite a marked increase in NO production during pregnancy, net Na retention occurs (Fig. 2) (3). During pregnancy, it is thus likely that NO acts to alter reflex control of renal output rather than directly influencing renal function. Indeed,
endogenous NO has been shown to suppress baroreceptor activity during pregnancy (18). There is also recent evidence that afferent discharge from atrial volume receptors is altered in pregnant rats (10). Our current findings lead us to suggest that the increased NO biosynthesis induced by pregnancy contributes to blunting the activity of the atrial volume receptors. Such might occur by changing the transducer properties of the volume receptors or by altering central processing of the afferent signals (8). This would result in blunted reflex inhibition of renal sympathetic nerve activity and, consequently, attenuated reduction in renal tubular Na reabsorption, the outcome of which would abet extracellular fluid volume expansion during pregnancy.

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REFERENCES