Differential effects of rat pregnancy on uterine and lung atrial natriuretic factor receptors

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THE BIOLOGICAL EFFECTS of atrial natriuretic factor (ANF) are produced by interaction with guanylyl cyclase (GC)-coupled plasma membrane receptors and generation of the second messenger guanosine 3',5'-cyclic monophosphate (cGMP) (3, 7). Three different ANF receptors have been identified and cloned (4, 8, 24, 27). Of these three ANF receptors, GC-A (5) and GC-B (4) are coupled to GC and mediate most of the biological effects of ANF (3, 7). ANF-C receptors are not linked to GC (8); they function as ligand clearance receptors (14) and are reported to be negatively coupled to the adenylate cyclase system (1). ANF GC-A receptors possess high affinity for both ANF and brain natriuretic peptide (BNP). GC-B receptors are preferentially activated by C-type natriuretic peptide (CNP) and to a lesser extent by ANF and BNP (4, 12, 24, 25).

ANF is a general smooth muscle relaxant (3) and exerts a tocolytic effect on the rat uterus (2, 20, 21). ANF receptors have been identified in human (9, 10) and rat (6, 22) uteri. Pregnancy has been shown to render refractoriness to the tocolytic activity of ANF (20, 21); ligand binding studies suggest that this refractoriness is caused by a downregulation of GC-linked ANF receptors (22). However, ligand-binding (9, 22) and cross-linking (6) studies cannot clearly distinguish between GC-A and GC-B receptors. Consequently, whether or not both GC-coupled ANF receptors are expressed in the uterus and modulated by pregnancy has not been unequivocally established. The present studies were therefore carried out to examine the modulation of mRNAs for different ANF receptor types in the rat uterus during pregnancy by ribonuclease protection assays and identify GC-A and GC-B receptors using antireceptor antibodies. As well, effects of ANF and CNP on cGMP production by uteri were determined. Lungs contain abundant ANF receptors (3, 18), which do not seem to be modified by pregnancy (17); lungs were therefore included in the study for comparison with data from uteri.

MATERIALS AND METHODS

Chemicals. The following agents were purchased: rat ANF (ANF-1-28) and rat CNP (CNP-22) from Peninsula (Belmont, CA); cGMP radioimmunoassay (RIA) kits from Amer- sham (Oakville, ON, Canada); 3-isobutyl-1-methylxanthine, bovine serum albumin (BSA), phenylmethylsulfonyl fluoride (PMSF), aprotinin, leupeptin, GTP, creatinine phosphate, and creatine phosphokinase from Sigma (St. Louis, MO). All other high-purity chemicals were purchased from Fisher (Montreal, QC, Canada).

Animals. Adult virgin female (175-200 g) and male (250-275 g) Sprague-Dawley rats (Charles River, St-Constant, QC, Canada) were used according to a protocol of the McGill University Animal Care Committee. Animals were housed on a 12:12-h light-dark schedule (lights on 0700–1900) at 22–25°C and 50–70% humidity and fed rat chow and tap water ad libidum. The presence of sperm in the vaginal washing after overnight housing with male rats denoted day 0 of pregnancy. Virgin, pregnant (7, 16, and 21 days), and postpartum (day 2) rats were decapitated, and uteri and lungs were quickly removed for the studies.

Ribonuclease protection assays. Uteri and lungs were snap-frozen in liquid nitrogen and stored at −80°C. Tissues were homogenized with a polytron in Trizol reagent (GIBCO, Burlington, ON, Canada), purified by phenol-chloroform extraction, and precipitated with ice-cold ethanol. The integrity of the RNA was assessed by formaldehyde-agarose gel electrophoresis followed by ethidium bromide staining, and the quantity was determined by absorbance at 260 nm. The preparation of sense and antisense primers used in the present study has been previously described in detail (17). The sense and antisense primers were made complementary to unique sequences spanning exon 3 and 6 of the receptor subtypes corresponding to the extracellular domain, which exhibit maximum heterogeneity (4, 5, 8, 27). 32P-labeled antisense RNA probes for ANF GC-A, GC-B, and C receptors were synthesized using an in vitro riboprobe transcription kit (Promega, Madison, WI). After digestion of the plasmids with Hind III for GC-A, BstI I for GC-B, and Dde I for the C receptors, the ribonuclease protection assays were performed as previously described (17).
**RESULTS**

Uterine ANF receptor mRNAs. mRNAs for all three ANF receptors were detected in uteri (Fig. 1). Pregnancy caused a significant decrease in GC-A (Fig. 1, A and D) and GC-B (Fig. 1, B and E) receptor mRNAs but no change (day 7 and day 21) or an increase (day 16) in ANF-C receptor mRNA (Fig. 1, C and F). The decrease in GC-B receptor mRNA was gestational age dependent, with the decrease being least on day 7 and maximal on day 21 of pregnancy (Fig. 1E). Both GC-A and GC-B receptor mRNAs recovered to virgin levels by postpartum day 2 (Fig. 1).

Uterine ANF receptor proteins. Both GC-A and GC-B ANF receptor proteins were abundantly expressed in uteri (Fig. 2). Pregnancy led to a significant decrease in uterine GC-A and GC-B receptor proteins (Fig. 2B).

Uterine GC activity. Both ANF (Fig. 3A) and CNP (Fig. 3B) caused a concentration-dependent increase in cGMP production by uterine membrane preparations; the maximal effect of CNP was greater than that of ANF (Fig. 3). Pregnancy caused a marked decrease in the effects of ANF and CNP on cGMP production; the maximal effects of both factors were significantly reduced in preparations from pregnant rat uteri.

Lung ANF receptor mRNAs. mRNAs for all the three ANF receptors (GC-A, GC-B, and C) were detected in lungs (Fig. 4, A-C). The abundance of ANF GC-A receptor mRNA was not altered by pregnancy (Fig. 4D). However, ANF GC-B receptor mRNA was significantly downregulated on days 7, 16, and 21 of gestation and did not return to levels in virgin rats by day 2 postpartum (Fig. 4E). In contrast to the effects on GC-B
receptors, pregnancy caused a significant increase in ANF-C receptor mRNA (Fig. 4F).

Lung ANF receptor proteins. Both ANF GC-A and GC-B receptor proteins were expressed in the lungs. GC-A receptor protein was approximately twofold more abundant than GC-B protein (data not shown). Pregnancy did not exert any significant effect on the abundance of GC-A and GC-B receptor proteins. Anti-ANF-C receptor antibodies were not available.

Lung GC activity. Both ANF and CNP caused a concentration-dependent increase in GC activity in lung preparations (data not shown). ANF was ~10-fold more potent than CNP, although the maximal effects of the two agents in preparations from virgin rats did not differ. Pregnancy did not significantly modify the effects of ANF and CNP on lung GC activity.

DISCUSSION

We have previously shown that pregnancy inhibited the tocolytic effects of ANF on rat uterus (20, 21) and decreased GC-linked ANF receptors (22). However, these studies did not identify whether one or both GC-coupled ANF receptors were downregulated. The primary purpose of the present study was to study in greater detail the modulation of uterine ANF receptors by pregnancy; lung ANF receptors were studied under identical conditions for comparison.

Fig. 2. Western blots of ANF GC-A (A, top) and GC-B (A, bottom) receptors in partially purified uterine membrane proteins from virgin and 16-day pregnant rats. A, top (GC-A) and bottom (GC-B): lane 1, ladder; lane 2, membrane proteins from virgin; lane 3, membrane proteins from 16-day pregnant rats. B: histogram of data (means ± SE) of 3–4 separate experiments. *Different (P < 0.05) from values for virgin controls. Western blots were prepared using polyclonal antireceptor antibodies in 1:500 dilution. Exposure time was 9 min.

Fig. 3. Effects of ANF and C-type natriuretic peptide (CNP) on particulate GC activity of uteri from virgin and 16-day pregnant rats. GC activity was determined by measuring formation of guanosine 3′,5′-cyclic monophosphate from a GTP-regenerating system in presence of 0.2 mM 3-isobutyl-1-methyl xanthine. Data are means ± SE of 4 separate experiments. *Different (P < 0.05) from corresponding values for virgin rats.
The presence of GC-A and GC-B ANF receptor mRNAs in human (10) and rat (6, 23) myometrium has been reported by other workers; our data confirm these findings. At the same time, the present study demonstrates a greater abundance of ANF-C receptor mRNA in the rat uterus than reported by others (23), possibly because the latter workers used human ANF-C receptor cDNA. To our knowledge, the present study for the first time demonstrates a significant modulation by rat pregnancy of uterine ANF receptor mRNAs and proteins and of ANF effects on GC activity and a reversal of these changes during postpartum. The downregulation of GC-B receptor mRNA was gestational age dependent and more marked than the decrease in GC-A mRNA. This differential effect of pregnancy on ANF receptor mRNAs implies variable sensitivities of receptor mRNAs to hormonal changes accompanying pregnancy. This suggestion is consistent with the observed upregulation of ANF-C receptors in uteri of 16-day pregnant rats and no changes at other gestational periods.

CG-linked ANF receptors have been identified in rat uterus by cross-linking studies (6). However, these studies do not clearly identify GC-A and GC-B receptor types because of comparable molecular size of these two receptors. The present study using Western analysis clearly shows the presence of both GC-A and GC-B receptor proteins in the rat uterus. The downregulation of both GC-A and GC-B receptor proteins by pregnancy supports our earlier conclusion based on ligand binding studies (22).

Antibodies against ANF-C receptors were not available, so the present studies do not clearly establish the presence of ANF-C receptors. The abundance of ANF-C receptor mRNA in rat uteri as found in the present study suggests that ANF-C receptors are expressed in uteri. An increase or no change in ANF-C receptor mRNA during pregnancy coupled with a decrease in GC-A and GC-B receptors would imply a relative increase in receptor-mediated clearance and a decrease in the tocolytic effects of ANF. The reversal of these changes during postpartum as found in this study is consistent with the restoration of the tocolytic effects of ANF soon after parturition (20).

Both ANF and CNP caused a concentration-dependent increase in cGMP production by uterine membrane preparations in conformity with other reports (6). However, these studies (6) used uterine strips and found CNP to be less effective than ANF in stimulating cGMP. In the present study using uterine membrane preparations, CNP was slightly less potent than ANF, but its maximal effect was greater than that of ANF. It is possible that the discrepancy between the present results and data of other workers (6) is due to different techniques employed to study effects on cGMP production. The results of the present study showing significant effect of CNP, which has a high affinity for GC-B receptors (12), are consistent with abundant expression of GC-B receptor mRNA and proteins in the uterus. Pregnancy caused a decrease in the effects of both ANF and CNP on cGMP production. Because pregnancy was associated with a downregulation of GC-A and GC-B receptors, it is reasonable to infer that the decrease in the production of the second messenger, cGMP, was due to a decrease in GC-linked receptors.

We detected mRNAs for all the three ANF receptors (GC-A, GC-B, and C) in rat lungs; these data confirm the findings of other workers (15, 26). At the same time, results of this study by Western analysis demonstrate
that both GC-coupled ANF receptors (GC-A and GC-B) are expressed in rat lungs, although GC-A receptors appear more abundant than GC-B receptors. Pregnancy did not modify mRNA for GC-A receptors. However, data were indicative of a decrease in mRNA for GC-B receptors and an increase in mRNA for ANF-C receptors. At the same time GC-A or GC-B receptor proteins did not appear to be modified by pregnancy. A lack of effect of pregnancy on ANF- or CNP-induced increase in lung GC activity further suggests that lung GC-coupled ANF receptors are not modulated by pregnancy, although pathological changes in plasma volume homeostasis such as in genetic cardiomyopathy in hamsters (15) and hypoxia in rats (11) are associated with changes in lung ANF receptors.

In summary, the present study demonstrates the presence of mRNAs for all the three ANF receptors (GC-A, GC-B, and C) in rat uteri and lungs and that pregnancy causes a downregulation of GC-A and GC-B receptors and a decrease in receptor-mediated effects on GC activity in the uteri but not in the lungs. A downregulation of uterine ANF receptors during pregnancy might be required physiologically to inhibit the tocolytic activity of ANF. The modulation of ANF receptors by pregnancy seems organ specific, since it is apparent in the uterus (present study), kidneys (17), adrenal zona glomerulosa cells (16) but not in lungs (this study) and aorta (21).

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