Gastrointestinal motility during pregnancy: role of nitric component of NANC nerves

SANGITA SHAH, ADRIAN HOBBS, RAJAN SINGH, JANIS CUEVAS, LOUIS J. IGNARRO, AND GAUTAM CHAUDHURI. Gastrointestinal motility during pregnancy: role of nitric component of NANC nerves. Am J Physiol Regulatory Integrative Comp Physiol 279: R1478–R1485, 2000.—This study evaluated whether increased release of nitric oxide (NO) from the nitric component of the nonadrenergic, noncholinergic (NANC) nerves may be partly responsible for the decrease in gastrointestinal motility observed during pregnancy. Segments of fundal strip, ileum, and colon were obtained from nonpregnant rats, rats in midpregnancy (days 9–11), and rats in late pregnancy (days 18–20). NANC activity was studied by assessing changes in tone after application of electric field stimulation (EFS). The role of NO was determined by observing the effects of EFS in the presence and absence of the NO synthase (NOS) inhibitor N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) and the reversibility of the effects of L-NAME by L-arginine. The magnitude of change in cGMP levels in the tissues after application of EFS was also assessed. Our studies indicate that there was increased magnitude of relaxation of isolated strips of rat gastric fundus and rat colon, after application of EFS to tissues obtained only from animals in late pregnancy. These results paralleled the changes in cGMP levels in tissues. NOS activity in the gastric fundus was significantly increased in animals in late pregnancy compared with nonpregnant controls. Our studies suggest that the delay in gastric emptying and increase in colonic transit time observed in rats during pregnancy may be caused in part by increased activity of the nitric component of the NANC nerves innervating these organs.

nitric oxide; gastrointestinal tract; gastrointestinal motility; pregnancy; estradiol; progesterone; nitric oxide synthase; cGMP; electric field stimulation; rats; nonpregnant; midpregnancy; late pregnancy; L-arginine; NO; transmural electrical field; cGMP

GASTROINTESTINAL DISORDERS constitute one of the most frequent symptoms during pregnancy (1). Decreased motility of the stomach (19, 22) leading to delay in gastric emptying time has been observed in pregnant women (11). This effect is probably caused by sex steroids, because postmenopausal women being treated with sex hormone replacement therapy have a decreased rate of gastric emptying of solids compared with men. In contrast, postmenopausal women without hormone replacement therapy have rates of gastric emptying of solids similar to those of men (17). Colonic transit time also increases during pregnancy. Contractility of colon muscle from rats in late pregnancy was significantly decreased compared with nonpregnant female rats (13). However, the issue of which of the sex steroids modulate these changes is controversial. Although progesterone has been implicated by numerous investigators as the hormone responsible for these changes (13, 24), this has not been universally accepted. Administration of estradiol and progesterone in combination or estradiol alone to ovariectomized rats slowed gastric emptying, whereas progesterone alone enhanced gastric emptying (9).

The precise mechanism by which sex steroids modulate the changes in gastrointestinal motility is also not known. Nitric oxide (NO) is released by the nonadrenergic, noncholinergic (NANC) nerves innervating the gastrointestinal tract after application of electric field stimulation (EFS) and mediates relaxation of the gastrointestinal smooth muscle (2, 3, 5, 21). There is increased NO synthase (NOS) expression in the gastrointestinal tissues during pregnancy (30, 31), and NO production and NOS expression are also increased by estradiol in various tissues (15, 30) including the gastrointestinal tract and neuronal tissues (30). Furthermore, increased production of NO is thought to mediate numerous physiological changes observed during pregnancy (23, 27).

We therefore decided to test the hypothesis that increased NO release from the nitric component of the NANC nerves may be partly responsible for the decrease in gastrointestinal motility observed during pregnancy. The pregnant rat was chosen as the animal model, because many of the gastrointestinal changes observed during pregnancy in rats are similar to those observed in humans (8, 9). The NANC nerves have also been well characterized in this species. Rats are relatively inexpensive, and their hormone profiles during pregnancy are well defined (20). Nonpregnant animals, animals in midpregnancy (9–11 days), and animals in late pregnancy (18–20 days) were used for this study, because their hormonal profiles are different (20) and would therefore allow us to indirectly assess the pos-

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sible role of estradiol and progesterone in modulating these changes.

MATERIALS AND METHODS

Animals

Virgin and pregnant female Sprague-Dawley rats (180–220 g, Harlan, Indianapolis, IN) were housed under conditions of controlled temperature and light cycle and were provided free access to food pellets and water. All animal experiments were performed after ethical approval was obtained from the Animal Research Committee of UCLA. Animals were euthanized by intramuscular injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) followed by exsanguination. The gastrointestinal tract was accessed by a midline incision of the abdomen. The stomach, a segment of the ileum, and a segment of the ascending colon just proximal to the cecum were removed and placed in Krebs bicarbonate solution (composition in mM: 118.0 NaCl, 4.7 KCl, 25.0 NaHCO₃, 1.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, and 11.5 glucose) gassed continuously with 95% O₂ and 5% CO₂.

Chemicals

ACh chloride, phenolamine, propranolol, atropine, 5-hydroxytryptamine (5-HT), N⁷-nitro-L-arginine methyl ester (L-NAME), triethanolamine hydrochloride (TEA-HCl), L-arginine-HCl, L-citrulline, pepstatin, leupeptin, L-valine dithiothreitol, sodium acetate, EDTA, EGTA, NDPAH, flavin adenine dinucleotide, flavin mononucleotide, tetrodotoxin were obtained from Sigma Chemical. L-[2, 3, 4, 5-³H]arginine HCl and cGMP and cAMP radioimmunoassay kits were obtained from Amer sham. Dowex AG I-X8 and Dowex A 5OW-X8 were obtained from Bio-Rad Laboratories (Richmond, CA).

Studies After Application of EFS to Strips of Fundus, Ileum, and Colon

Studies with fundus. For preparation of the rat fundal strips (28), the fundal portion of the stomach was dissected free, cut along the antimesenteric surface, and rinsed of any residual contents with Krebs buffer. Longitudinal strips (5 mm × 10 mm) were cut and placed in a dish containing freshly prepared Krebs buffer. The tissues were suspended in a 25-ml water-jacketed (37°C) organ bath containing Krebs bicarbonate solution gassed continuously with 95% O₂-5% CO₂. The appropriate resting tension for strips was determined in initial experiments. Strips were placed under progressive increments of tension, and contractile responses to KCl (120 mM in Krebs bicarbonate solution) were measured under the various resting tension conditions. Optimal length-tension relationships were achieved with resting tensions of 1 g for the fundal strips. Therefore, a resting tension of 1 g was applied to the tissues, and changes in tension were recorded with a Grass FT03 force displacement transducer attached to a Soltec chart recorder. Tissues were incubated with atropine (10 μM), phenolamine (10 μM), and propranolol (10 μM) for 30 min to ensure blockade of adrenergic and cholinergic receptors. To observe relaxations, tone was raised to 60–80% of maximal contraction produced by 120 mM KCl by addition of 5-HT to a final concentration of 30 μM. After maintenance of active tone for 30 min, EFS was performed with two parallel platinum electrodes connected to a current amplifier and stimulator (SD9, Grass Instruments) placed 4–5 mm apart surrounding the middle portion of the strip. Field stimulation was applied as 0.5-ms pulse width and supramaximal voltage (40 V) during each 10-s train of stimulation throughout the experiment. In preliminary experiments, these parameters were selected after construction of frequency-response curves at various voltages and then observing the absence of relaxation at the parameters used when EFS was applied in the presence of tetrodotoxin. Stimulation was delivered at varying frequencies (1, 2, 5, 10, 20, and 40 Hz), and the resulting changes in response were measured. The same protocol was repeated after the addition of L-NAME (100 μM) either alone or in combination with L-arginine (1 mM).

Studies with ileum. The ileum was rinsed intraluminally with Krebs bicarbonate solution to remove any food contents. A 3- to 4-cm segment of the ileum was suspended in a 25-ml water-jacketed organ bath as described in Studies with fundus. Optimal length-tension relationships were achieved with resting tension of 1 g for a segment of the ileum, and hence, a resting tension of 1 g was applied to the tissues, which were allowed to equilibrate for 30 min (recorded by a force displacement transducer as for fundus). Two parallel platinum wire electrodes were positioned on either side of the tissue, and stimulations were carried out using the same parameters as stated for fundus studies. It was difficult to maintain a sustained active tone in the ileum with any of the substances known to cause contraction of the gastrointestinal tract except carbachol. We therefore used a technique to study the role of NANC nerves in the ileum previously described by other investigators (4). The role of NANC innervation to the ileum was assessed indirectly by initially assessing the contraction of the ileum after application of EFS only in the presence of adrenergic blockers (as used for studies with fundal strip) and then reassessing the contraction after application of EFS in the presence of L-NAME (100 μM). These frequency-dependent contractions were then normalized to the maximal contraction obtained by ACh (100 μM) and compared between groups. EFS was applied at varying frequencies (1, 2, 5, 10, and 15 Hz). This protocol was repeated after the addition of L-NAME (100 μM) either alone or in combination with L-arginine (1 mM).

Studies with colon. The colon was rinsed intraluminally with Krebs bicarbonate solution to remove any contents. A 3- to 4-cm segment of the colon was suspended in a 25-ml water-jacketed organ bath. Optimal length-tension relationships were achieved with resting tensions of 2 g for segments of the colon, and hence, a resting tension of 2 g was applied to the tissues, which were allowed to equilibrate for 30 min (recorded by a force displacement transducer as for fundus and ileum). Tissues were incubated with atropine (10 μM), phenolamine (10 μM), and propranolol (10 μM) for 30 min to block the adrenergic and cholinergic receptors. Two parallel platinum wire electrodes were positioned on either side of the tissue, and stimulations were carried out using the parameters as stated for fundus and ileum. As with the ileal tissue, it was difficult to maintain a consistent active tone of segments of colon with any available pharmacological agents except carbachol. In these tissues, application of EFS resulted in a quiescence of the intrinsic rhythmic activity of the tissue. EFS was applied at varying frequencies (1, 5, 10, 15, 30, and 45 Hz), and the responses were measured as a function of the length of time of quiescence. This protocol was repeated in a similar manner after preincubation of the tissues with L-NAME (100 μM) either alone or in combination with L-arginine (1 mM).
**Determination of cGMP Levels**

cGMP determinations were made in strips of gastric fundus, strips of ileum, and colon set up in organ baths as described in Studies After Application of EFS to Strips of Fundus, Ileum, and Colon. The time at which peak levels of cGMP occurred after application of EFS was determined. The tissues were stimulated at 45 Hz for 10 s, and the tone was monitored until the tissues were freeze-clamped at various time points (0, 15, 30, 45, and 60 s). The use of drop-away bath, freeze-clamping of tissues, preparation and extraction of tissues for cyclic nucleotide determinations, and radioimmunoassay procedures has been described previously (12, 14, 18). Recoveries of standard amounts of added cGMP were determined, and values ranged from 95% to 102%. All samples were run in a single assay to minimize problems with interassay variation.

**NOS Assay**

The conversion of L-arginine to L-citrulline (26) was used to assess NOS activity in homogenates of gastric fundus. A 20% (wt/vol) homogenate of gastric fundus was prepared in 50 mM TEA-HCl, pH 7.4, containing 0.1 mM EGTA, 0.1 mM EDTA, 0.5 mM dithiothreitol, 1 μM pepstatin A, and 2 μM leupeptin at 4°C. The homogenate was centrifuged at 20,000 g for 60 min at 4°C, and the supernatant was used to assay NOS activity. NOS activity was determined by measuring the formation of L-[3H]citrulline from L-[1-3H]arginine. Enzymatic reactions were conducted at 37°C for 10 min in 50 mM TEA-HCl, pH 7.4, containing 50 μM L-arginine (77 Ci/mmol, with ~20,000 cpm of L-[2,3,4,5-3H]arginine HCl), 100 μM NADPH, 10 μM tetrahydrobiopterin, 10 μM flavin mononucleotide, 2 mM CaCl₂, 1 μg of calmodulin, and 0.2–0.4 mg of supernatant protein in a final incubation volume of 100 μl. Ca²⁺-independent NOS activity was measured in the absence of Ca²⁺ and calmodulin and in the presence of 2 mM EDTA and 2 mM EGTA. Ca²⁺-dependent NOS activity was obtained by subtracting the Ca²⁺-independent NOS activity from the total NOS activity. L-[2,3,4,5-3H]arginine HCl was purified by anion-exchange chromatography on columns of Dowex AG 1-X8 (OH⁻ form prepared from the acetate form, 100–200 mesh) to remove traces of contaminating [3H]citrulline. Enzymatic reactions were terminated by addition of 2 ml of ice-cold buffer (20 mM sodium acetate, pH 5.5, containing 1 mM L-citrulline, 2 mM EDTA, and 0.2 mM EGTA), and samples were loaded onto columns (1-cm diameter) containing 1 ml of Dowex AG 50W-X8 (Na⁺ form prepared from H⁺ form) that had been preequilibrated with stop buffer for chromatography. After 2 ml of eluate were collected in a test tube, each column was washed again with 2 ml of water and collected in the same test tube. Aquasol-2 (12 ml) was added to one-half (2 ml) of the final eluate, and the samples were counted in a liquid scintillation spectrometer (model LS 3801, Beckman).

**Data Analysis**

Values are means ± SE obtained from five to seven animals in each group. Values between groups were compared using two-way analysis of variance with Duncan’s t-range or Student’s t-test where appropriate.

**RESULTS**

**Study 1. Studies After Application of EFS to Strips of Fundus, Ileum, and Colon**

**Studies with fundal strip.** Application of EFS resulted in a transient decrease in tone, and the magnitude of relaxation was frequency dependent. The relaxant response started at the time of application of EFS. The time taken for the tone to return to baseline correlated directly with the intensity of the frequency of the stimulus. Figure 1 shows representative traces obtained after application of EFS to a fundal strip obtained from a nonpregnant and a late pregnant animal. Relaxant responses in tissues obtained from late pregnant animals were 50% greater than those of nonpregnant animals, whereas tissues from midpregnant animals responded in a manner similar to those nonpregnant animals (Fig. 2). In some experiments, pretreatment with tetrodotoxin (1 μM) in the tissue bath for 30 min abolished all responses to EFS (data not shown). We further evaluated whether NO was one of the mediators involved in the increased magnitude of relaxation after application of EFS. This was assessed by observing the magnitude of relaxation in the absence and presence (100 μM) of L-NAME alone (after incubation of tissue with L-NAME for at least 15 min) or in the presence of L-NAME (100 μM) and either L- or D-arginine (1 mM). In both nonpregnant and late pregnant animals, L-NAME attenuated the relaxant responses after application of EFS, and this inhibition

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**Fig. 1.** Representative traces of the relaxant responses to electrical field stimulation (EFS; 20 Hz, 0.5-ms pulse width, 40 V, 10 s) of rat gastric fundus obtained from a nonpregnant (top) and a late pregnant (bottom) animal. In tissues obtained from late pregnant animals, the magnitude of relaxation after EFS was significantly greater compared with those obtained from nonpregnant animals. Tissues were incubated with atropine, phentolamine, and propranolol (10 μM) and precontracted with 5-hydroxytryptamine (5-HT; 30 μM).
was greater at lower frequencies in both nonpregnant and pregnant animals. L-Arginine (Fig. 3) but not D-arginine (data not shown) significantly reversed the effects of L-NAME.

Studies with ileum. Ileal segments obtained from nonpregnant, midpregnant, and late pregnant rats contracted after application of EFS (1–15 Hz; 10-s trains), and this was frequency dependent. The contractile effects after application of EFS were most likely caused by the release of ACh resulting from stimulation of cholinergic nerves (because atropine was not added to the muscle bath). However, the simultaneous release of relaxant mediators from the NANC nerves, like NO, attenuates the contraction because of their functional antagonism. The modulating role of NO simultaneously released along with ACh in this contractile effect was indirectly assessed in tissues obtained from the three different groups of animals by reassessing the magnitude of contraction in the presence of L-NAME and comparing the values obtained in the absence of L-NAME. There was a significant increase in the contractile response in the presence of L-NAME after application of EFS in tissues obtained from all three groups of animals. Unlike the fundus, no significant differences in the responses were observed in segments of the ileum obtained from the different groups of animals in the presence or absence of L-NAME (data not shown). In some experiments, preincubation of the tissues for 30 min with tetrodotoxin (1 μM) abolished all responses to EFS, suggesting a neurogenic response to EFS (data not shown). Unlike the experiments with the gastric fundus, the onset of the contractile response in the ileum was not observed during EFS but occurred only after the stimulation was terminated, whereas in the presence of L-NAME, the contractile response occurred at the time of application of EFS (Fig. 4), suggesting that NO caused the increased latency of response after application of EFS in the absence of the NOS inhibitor.

Studies with colon. Application of EFS resulted in a frequency-dependent inhibition of spontaneous rhythmicity that remained for varying lengths of time depending on the magnitude of stimulation. The duration of this inhibitory effect after application of EFS greatly increased in tissues obtained from animals in late pregnancy at all frequencies studied (Fig. 5). Prior incubation of the tissue with L-NAME resulted in an increase in basal spontaneous motility and a decrease in the duration of inhibitory response after application of EFS (1–45 Hz; 10-s trains). Addition of L-arginine (1 mM) before L-NAME significantly reversed the responses observed with L-NAME alone (data not shown).

Study 2. Studies Assessing Magnitude of Relaxant Responses of Strips of Fundus, Ileum, and Colon to DEA-NO, an NO Donor

In these experiments, the strips of fundus, ileum, and colon were set up in isolated organ baths and phentolamine (10 μM), propranolol (10 μM), and L-NAME were added to oxygenated Krebs bicarbonate solution. Active tone was induced by carbachol (10 μM). Concentration-response curves were generated using sodium(Z)-1-(N,N-diethylamino)-diazen-1-ium-1,2-diolate (DEA-NO; 10⁻⁸ M to 10⁻⁴ M). The EC₅₀ for the fundal strip and segments of the ileum and colon obtained from nonpregnant animals were 60 ± 15, 20 ± 5, and 45 ± 8 μM, respectively. The corresponding EC₅₀ values for the fundal strip and segments of the ileum and colon obtained from pregnant animals were 73 ± 20, 14 ± 5, and 57 ± 7 μM, respectively. Comparison of the EC₅₀ values for DEA-NO showed no
significant difference between tissues obtained from nonpregnant and late pregnant animals.

**Study 3. Studies Assessing Changes in cGMP Levels After Application of EFS to Strips of Fundus, Ileum, and Colon**

**Studies with fundal strips.** After induction of active tone with 5-HT (30 μM), EFS was applied for 10-s trains at maximal frequency (40 Hz) and tissues were frozen after 15 s as described previously (12, 14, 18). This time point was selected because in preliminary experiments we observed maximal cGMP levels 15 s after initiation of EFS. A fivefold increase in cGMP levels over basal values was observed in tissues obtained from late pregnant animals vs. a 2.5-fold increase in those obtained from nonpregnant animals. Incubation of tissues with L-NAME (100 μM) for 30 min before application of EFS inhibited the increase in cGMP (Fig. 6).

**Studies with ileum.** Application of EFS to ileal segments obtained from nonpregnant and late pregnant animals increased cGMP levels in both groups, and there was no significant difference in the magnitude of increase in cGMP levels between the two groups. Application of EFS after preincubation of tissues with

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**Fig. 4.** Representative traces of the contractile responses to EFS (1–15 Hz) of ileum obtained from an animal in late pregnancy after application of EFS. These frequency-dependent contractions were then normalized to the maximal contraction obtained by ACh (100 μM). A: control. B: in the presence of L-NAME (100 μM). Note the latency period for onset of contraction of the control tissues and its abolition in the presence of L-NAME.

**Fig. 5.** Frequency-response curves of segments of rat colon obtained from nonpregnant animals and from animals in mid- and late pregnancy assessing the lag phase after application of EFS (1–30 Hz; 10-s trains). Responses were measured as duration of decreased motility after application of EFS. Each point represents mean values ± SE from at least 5 animals. *Significant difference from nonpregnant animals (P < 0.05).

**Fig. 6.** cGMP levels after application (40 Hz) of EFS in the absence and presence of L-NAME (100 μM) to rat fundal strips obtained from nonpregnant animals and from animals in late pregnancy. Each point represents mean values ± SE obtained from at least 5 animals. *Significant difference from basal values (P < 0.05); †significant difference (P < 0.05) from nonpregnant controls after application of EFS; **significant difference (P < 0.05) from values obtained after application of EFS in the absence of L-NAME.
L-NAME decreased cGMP levels to a similar extent in both groups (data not shown).

Studies with colon. Isolation of tissues 15 s after application of EFS (45 Hz; 10-s trains) resulted in an 11-fold increase in cGMP levels over basal values; this was significantly greater in tissues obtained from late pregnant animals compared with those obtained from nonpregnant animals (7-fold) (Fig. 7). Preincubation of tissues with L-NAME (100 μM) inhibited the increase in cGMP observed in its absence in both groups.

Study 4. Effect of Pregnancy on NOS Activity in Fundus

There was a significant increase in Ca$^{2+}$-dependent NOS activity, as measured by conversion of L-arginine to L-citrulline, in the fundus obtained from animals in late pregnancy compared with that obtained from non-pregnant animals (Fig. 8). The values for the Ca$^{2+}$-independent NOS activity were barely above background values, and there was no significant difference among the groups (data not shown).

DISCUSSION

The primary objective of this study was to assess whether there is an increase in NO release from the nitrergic component of the NANC nerves of the gastrointestinal tract during pregnancy. Because the hormone profiles change in different phases of pregnancy, we elected to study animals in mid- and late pregnancy and compare the findings with nonpregnant animals. There was increased magnitude of relaxation to EFS of isolated strips of rat gastric fundus and rat colon, but not of the ileum, obtained from animals in late pregnancy, but not from animals in midpregnancy, compared with nonpregnant animals. The relaxation after application of EFS was caused by stimulation of the NANC nerves, because the effects were observed in the presence of adrenergic and cholinergic receptor blockers and the responses were abolished in the presence of tetrodotoxin. Application of EFS at low frequencies (1–20 Hz) to precontracted strips of gastric fundus relaxed the strips only during the period of transmural stimulation, and the tone returned to initial levels immediately after cessation of EFS. In contrast, EFS at higher frequencies (≥20 Hz) induced relaxation that persisted for some time even after cessation of the stimulation.

The different patterns of relaxation of the fundal strip that were observed at low and high frequencies are similar to those reported by other investigators (3). It has been demonstrated that both NO (2–4, 21) and vasoactive intestinal polypeptide (VIP) (21) are involved in the inhibitory NANC innervations of the rat gastric fundus, and NOS has been shown to be colocalized with VIP in the myenteric nerve endings (10). The differences in the duration of relaxant responses observed at lower and higher frequencies of EFS suggest that different mediators may be involved. The relaxant response to NANC stimulation at low frequencies was of a short duration, and the tone returned to baseline almost immediately, suggesting the release of a mediator with a very short half-life such as NO. On the other hand, application of EFS at higher frequencies led to a more prolonged relaxant response with a slow recovery, suggesting that a long-acting mediator like VIP may be involved. In fundal strips obtained from pregnant animals, the magnitude of relaxant responses to EFS was much greater at lower frequencies (1–20 Hz) than at higher frequencies. This suggested an increased release of NO from the NANC nerves...
during late pregnancy and was not caused by increased sensitivity to NO, because no change in the response to DEA-NO was observed between any of the groups. Furthermore, L-NAME, an inhibitor of NO synthesis, decreased the magnitude of relaxation of fundal strips, and this attenuation was greatest at lower frequencies rather than at higher frequencies. This strongly suggests the possibility that NO is an important mediator responsible for the increased magnitude of gastric relaxation after application of EFS observed in pregnant animals. The increased Ca^{2+}-dependent NOS activity in the fundus of pregnant rats compared with that obtained from nonpregnant animals correlates positively with the increased relaxation to EFS in pregnant animals.

A likely mechanism by which NO causes relaxation of the fundal strip is the stimulation of soluble guanylate cyclase resulting in cGMP formation (12, 14, 18). The greater magnitude of increase in cGMP after application of EFS to fundal strips obtained from rats in late pregnancy, and inhibition of this increase by L-NAME, support a role for the NO-cGMP pathway in the delayed gastric emptying in late pregnancy. This could be caused by an increase in the release of NO after application of EFS, an increased sensitivity of guanylate cyclase to NO, or both. Because no increase in sensitivity of guanylate cyclase to the NO donor DEA-NO was observed in our study, one can conclude that the effects observed in rats in late pregnancy were most likely caused by increased synthesis and release of NO from the NANC nerves.

In studies with the proximal colon, we observed basal rhythmic contractions that increased markedly after incubation with L-NAME. This indicates that the inherent rhythmic motility of the colon is modulated by a basal release of NO. After EFS, there was a transient frequency-dependent decrease in tone and cessation of rhythmic activity, a lag phase after which the tone returned to prestimulation level with resumption of spontaneous activity. This effect was predominantly NO mediated, because it was significantly reduced by L-NAME. This lag phase was significantly longer in segments of colon obtained from animals in late pregnancy compared with those obtained from animals in midpregnancy or nonpregnant animals. The fact that the response to DEA-NO was similar on segments from colon obtained from the various groups indicates that this increase in lag phase seen after EFS in colon obtained from animals in late pregnancy was most likely caused by an increased synthesis and release of NO. It therefore appears that the decrease in motility of the fundus and colon associated with pregnancy may be caused at least in part by increased release of NO from the NANC nerves innervating these organs.

In studies with the ileum, no cholinergic receptor blockers were used, and the contribution of NO released after application of EFS was indirectly assessed by observing an increase in the magnitude of contraction in the presence of L-NAME. In this set of experiments, the contractile effect was observed only after discontinuation of the EFS, whereas in the presence of L-NAME, the contractile effect occurred during application of EFS. This indicates that, during application of EFS, there was release of NO, and this in some manner inhibited the release or action of the motor transmitter ACh during application of EFS. This phenomenon was reported previously by other investigators (6, 16). In this study, we did not assess the precise mechanism involved. However, it has been suggested by other investigators that this may be caused by NO acting at either a prejunctional (16) or a postjunctional (6) site of the cholinergic neurons. A similar modulation of sympathetic responses by nitrergic transmission in the rabbit anococcygeus muscle and rabbit and human corpus cavernosum has also been described (7).

Results from our studies may have important physiological and clinical implications. During pregnancy, there is delayed gastric emptying (11), and rats in diestrus had slower gastric emptying than ovariectomized rats (8), suggesting the modulating role of sex steroids in this phenomenon. Similarly, during pregnancy, colonic transit time also increases. Contractility of colon muscle from rats in late pregnancy was significantly decreased compared with that in nonpregnant female rats (25). The increased colonic transit time is caused by altered sex steroid levels, because pregnant rats had mean colonic transit times essentially identical to those of animals pretreated with hormones (13). On the other hand, the effect of pregnancy on small bowel transit time is controversial. Small bowel transit time is greater during pregnancy, when estradiol and progesterone levels are substantially increased, compared with the postpartum period, when hormone levels have returned to normal values (29). However, no differences in intestinal transit time were observed between pregnant and nonpregnant rats (25) of the species utilized in this study. Our observation that there is increased release of NO after NANC nerve stimulation in the fundus and colon but not in the ileum correlates well with in vivo studies of transit time in different segments of the bowel in this species.

Our studies did not address whether the pregnancy-related changes in the nitrergic component of the NANC activity in tissues obtained from animals in late pregnancy were mediated by sex steroids or which of the sex steroids played a dominant role. In rats, progesterone levels increase throughout gestation and plateau at midpregnancy. However, estradiol levels begin to increase only in midpregnancy and continue to rise until parturition (20). This would suggest that estradiol or a combination of estradiol and progesterone, but not progesterone alone, is responsible for the effects observed, because the increased NO production during pregnancy was observed in tissues obtained from animals in late pregnancy but not from those obtained from animals in midpregnancy. The possibil-
ity that estradiol, but not progesterone, may play a more important role in increasing NOS activity in NANC nerves is supported by data from the studies of other investigators, in which estradiol but not progesterone increased nitric oxide production in various tissues (15, 30) including neuronal tissue and the gastrointestinal tract (30). Further work is in progress to identify the sex steroids responsible for these changes and the cellular and molecular mechanisms involved.

**Perspectives**

Increased release of NO from NANC nerves may be modulated by estradiol and/or progesterone and may explain many of the gastrointestinal changes such as esophageal reflux, delayed gastric emptying, and constipation experienced by women during pregnancy.

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