Action of progesterone on contractile activity of isolated gastric strips in rats

Fang Wang, Tian-Zhen Zheng, Wei Li, Song-Yi Qu, Di-Ying He

INTRODUCTION
Nausea and vomiting are extremely common complaints of pregnancy and may precede even the patients are aware that she is pregnant[1-4]. However, its mechanism is poorly understood. The questions of whether gastric emptying of solids and liquids differs in men and women and whether emptying is influenced by the action of sex hormones on gastric smooth muscle remain unresolved[5-8]. Whether gastric emptying of solids and liquids differs in women during the menstrual cycle is controversial[9-12]. The results of several clinical and physiological studies have suggested that the aforementioned complaints of pregnancy may be related, at least in part, to decrease of resting tension within the lower esophageal sphincter and changes in gastric motility[13-15]. The fact that a high serum sex hormone concentration is the characteristic of pregnancy tempts researchers to investigate the hormonal factor associated with gastrointestinal dysmotility. However, so far, the effect of pregnancy and sex hormone on gastric motility remains controversial. We studied the action of progesterone on the gastric strips in rats and explored the possible mechanism concerned.

MATERIALS AND METHODS

Materials
Progesterone, purchased from sigma, was dissolved and diluted in 1, 2-propanecol; hexamethonium and Nw-Nitro-L-Arginine (L-NNA), Sigma; indomethacin, Jiangsu Taicang Pharmaceutical Factory; propranolol, Beijing Thirteen Pharmaceutical Factory; Phentolamine, Beijing Thirteen Pharmaceutical Factory; 1, 2-propanecol, Tianjing Chemical Pharmaceutical Factory; 1, 2-propanecol, Tianjing Chemical Factory; Krebs buffer solution [(mmol·L\(^{-1}\)]: NaCl 120. 6, KCl 5. 9, NaH\(_2\)PO\(_4\) 1. 2, MgCl\(_2\) 1. 2, NaHCO\(_3\) 15. 4, CaCl\(_2\) 2. 5, Ca\(_2\)(H\(_2\)O\(_4\)) 11. 5, pHe=7.4)].

Methods
Wistar rats were fasted with free access to water for 24 h, and sacrificed to remove whole stomach. Then, the stomach was opened along the great curvature, and rinsed with Krebs solution. The stomach was pinned on a wax block with mucosa side up, and the mucosal layer was gently rubbed with a tweezers. Parellel to either the circular or the longitudinal fibers, muscle strips were cut from fundus, body, antrum and pylorus. Each muscle strip was suspended in a tissue chamber containing 5 mL Krebs solution. Then the motility of gastric strips in tissue chambers were simultaneously recorded. The preparations were subjected to 1 g load tension and washed with 5 mL Krebs solution every 20 min. After 1 h equilibration, progesterone or antagonists were added in the tissue chamber separately. The antagonists were added 3 min before using progesterone (50 µmol·L\(^{-1}\)).

RESULTS:
Progesterone decreased the resting tension of fundus and body longitudinal muscle (LM) (P<0.05). It inhibited the mean contractile amplitude of body and antrum LM and circular muscle (CM), and the motility index of pyloric CM (P<0.05). The inhibition of progesterone on the mean contractile amplitude could be partially blocked by phentolamine in LM of the stomach body (the mean contractile amplitude of body LM decreased from -7.5±5.5 to -5.2±4.5 P=0.01), and by phentolamine or indomethacin in CM of body (the inhibition of progesterone on the mean contractile amplitude of body CM decreased from -5.6±3.0 to -3.6±2.7 by phentolamine and from -5.6±3.0 to -3.5±2.5 by indomethacin, P=0.01). Hexamethonium, propranolol and L-NNA (inhibitor of NO synthetase) didn’t affect the action of progesterone (P>0.05).

CONCLUSION:
The study suggested that progesterone can inhibit the contractile activity of isolated gastric strips in rats and the mechanism seems to be a direct one except that the action on gastric body is mediated through prostaglandin and adrenergic \(\alpha\) receptor partly.

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solution every 20 min. After 1 h equilibration, progesterone (5, 10, 50 μmol·L\(^{-1}\)) or antagonist was added in the tissue chamber (all were the final concentration) separately 3 min before using progesterone (50 μmol·L\(^{-1}\))[16-20].

**Analysis of data**

We measured the resting tension of all strips, the mean contractile amplitude of body and antrum strips, and the motility index (MI= Σ[amplitude×duration]) of pyloric strip. Frequencies of contraction were determined by counting the contraction waves. Values of the results was presented as ±s. Statistical significances were measured by t test[16,17].

**RESULTS**

**Effect of progesterone on spontaneous contraction of gastric strips**

Progesterone significantly decreased the resting tension of fundus and body LM (Table 1). It decreased the mean contractile amplitude of body and antrum, and the motility index of pylorus (Table 2). However it didn’t influence the gastric contractile frequency (P>0.05).

| Progesterone μmol·L\(^{-1}\) | Fundus | Body | Antrum | Pylorus |
|-------------------------------|--------|------|--------|---------|
|                              | LM     | CM   | LM     | CM      |
| 5                             | -0.08±0.12\(a\) | -0.006±0.08 | 0       | 0.01±0.05 | 0       |
| 10                            | -0.08±0.08\(b\) | -0.05±0.11 | -0.03±0.06 | 0.04±0.09 | 0       |
| 50                            | -0.09±0.06\(d\) | -0.12±0.04 | 0.01±0.15 | 0.02±0.04 | 0 -0.03±0.10 |

The values were expressed as differences in resting tension between 3 min before and after the addition of progesterone (5, 10 and 50 μmol·L\(^{-1}\)) (The same in Tab 2). The resting tension of each strip in control (progesterone 0 μmol·L\(^{-1}\)) or antagonist was added 3 min before admistration (Pr: progesterone, I: indomethacin, Ph: phentolamine).

**Effect of antagonists added progesterone on spontaneous contraction of gastric strips**

Hexamethonium (10 μmol·L\(^{-1}\)), L-NNA (100 μmol·L\(^{-1}\)) or propranolol (1 μmol·L\(^{-1}\)) added 3 min before admistration progesterone didn’t influence the decreasing effect of progesterone on the gastric strips in rats (P>0.05), but phentolamine (1 μmol·L\(^{-1}\)) partly blocked its effect on the mean contractile amplitude of body LM and CM, and indomethacin (10 μmol·L\(^{-1}\)) also decreased the effect on the mean contractile amplitude of body CM (Table 3).

**DISCUSSION**

It has been shown from humans and animals that pregnancy is associated with alternations in the motor activity of the gastrointestinal tract, such as decreased gallbladder contractility and lower esophageal sphincter pressure, reduced gastric emptying, small intestine and colonic transis[13-15,21-29]. Although the factors responsible for the impaired gastric motility are obscure, there is evidence to suggest that pregnancy is associated with disturbances in the myoelectric and mechanical properties of gastrointestinal smooth muscle.

| Progesterone μmol·L\(^{-1}\) | Contractile amplitude/mm | Motility index/cm·s\(^{-1}\) |
|-------------------------------|---------------------------|-------------------------------|
|                              | Body | Antrum |            | Body | Antrum | Pylorus |
| 5                             | 13.4±17.0 | 12.0±13.2 | 13.6±8.6 | 14.1±15.0 | 92.4±16.2 |
| 10                            | 0.2±0.4 | -0.9±0.10 | -0.5±0.8 | -0.8±1.7 | 1.7±0.8 |
| 50                            | 12.8±17.6 | 11.2±14.0 | 14.0±7.1 | 13.6±12.9 | 98.5±20.0 |
|                               | -2.1±3.8 | -1.5±0.6 | -1.8±0.6 | -3.1±1.8 | -22.5±16.6 |
|                               | 12.0±16.9 | 11.8±12.9 | 12.6±8.0 | 12.6±14.8 | 110.2±22.8 |

Phentolamine (1 μmol·L\(^{-1}\)) or indomethacin (10 μmol·L\(^{-1}\)) was added 3 min before the addition of progesterone (50 μmol·L\(^{-1}\)). The values were expressed as differences in contractile amplitude of body before and after administration of progesterone (Pr) and indomethacin (I) or phentolamine (Ph). The values were expressed as differences in contractile amplitude of body before and after administration of progesterone (Pr) and indomethacin (I) or phentolamine (Ph). The values were expressed as differences in contractile amplitude of body before and after administration of progesterone (Pr) and indomethacin (I) or phentolamine (Ph). The values were expressed as differences in contractile amplitude of body before and after administration of progesterone (Pr) and indomethacin (I) or phentolamine (Ph). The values were expressed as differences in contractile amplitude of body before and after administration of progesterone (Pr) and indomethacin (I) or phentolamine (Ph).
In our study, progesterone decreased the resting tension of fundus, which might be a cause of changed gastric motility during pregnancy. It had been agreed that decreased fundic resting tension mainly influenced the gastric emptying of liquids. Ryan also reported[30] that pregnancy was associated with decreased gastric emptying of liquids in the guinea pig. The observation in our study that hexamethionium, L-NNA and propranolol didn’t influence the effect of progesterone suggesting that the action of progesterone was not mediated via NO, β or N receptors. Since phentolamine blocked partly the effect of body LM and CM, and indomethacin decreased that of body CM showed that the effect of the hormone on body LM partly via a receptor, and on body CM via prostaglandin and a receptor. In addition, the effect of progesterone might act on gastric smooth muscle cells directly. Progesterone receptor had been found in normal human gastric tissues. Another evidence was addition of progesterone to isolated denervated gallbladder muscle strips inhibited contraction in response to both acetylcholine or cholecystokinin[34].

Parkman reported[29] that spontaneous and bethanechol induced phasic antrum contraction of pregnant guinea pigs were significantly reduced in force compared with control virgin animals, and intracellular electrical recordings were obtained from antral smooth muscle cells to investigate the mechanism of the decreased contractility of antral smooth muscle during pregnancy. The results showed that there were similar resting membrane potentials, slow wave frequency and slow wave duration vs those of the control, but the upstroke amplitude, plateau amplitude and number of spike per slow wave decreased significantly. Further study suggested that the decreased force of spontaneous antral contractions was associated with a reduction in the underlying electrical slow wave depolarization. Electrogastrogram recordings also suggested that gastric dysrhythmias were objective pathophysiologic event associated with symptoms of nausea and vomiting during pregnancy[14,32,33].

Exogeneous progesterone also inhibited the myoelectric and mechanical activity of gastrointestinal smooth muscle. Electrical spike potentials recorded from chronically implanted electrodes in the antrum and jejunum of ovariecotomized dogs by Milenory decreased after 4 d of progesterone addition (2 mg·kg⁻¹·d⁻¹) and the propagation velocity of the basic electrical rhythm from the antral region of the progesterone-treated animals also decreased[31]. In another example, progesterone had been shown to reduce the propagation velocity of gastrointestinal slow waves possibly by decreasing the degree of electrical coupling between smooth muscle cells[35]. Dysrhythmias were also induced in healthy, nonpregnant women by administration of progesterone in the dose that reproduces plasma level seen in pregnancy. The above results suggested that the inhibitory effect of progesterone on the gastric smooth muscle may contribute to the gastric dysmotility during pregnancy.

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