Human chorionic gonadotropin regulates gastric emptying in ovariectomized rats

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Abstract

Prolongation of gastrointestinal transit resulting in nausea and vomiting in pregnancy (NVP) is the most common phenomenon during the first trimester of pregnancy. Increased human chorionic gonadotropin (hCG) concentration during the first trimester is the most likely cause of NVP. The aim of this study was to investigate the effect of hCG on gastrointestinal transit and plasma concentrations of cholecystokinin (CCK) in ovariectomized (Ovx) rats. I.p. injection of hCG was used to evaluate the dose effect of hCG on gastrointestinal transit in Ovx rats. The CCK antagonist lorglumide was used to clarify the role of CCK in regulating gastrointestinal transit. Gastrointestinal transit was assessed 15 min after intragastric gavage of a mixture of 10% charcoal and Na₂⁵¹CrO₄ (0.5 µCi/ml). After i.p. administration of hCG, gastric emptying was inhibited in Ovx rats, but intestinal transit was not affected. Plasma CCK concentrations were increased in a dose-dependent manner after hCG treatment, and gastric emptying showed a significant negative correlation with CCK concentrations ($P=0.01$, $r^2=-0.5104$). Peripheral administration (i.p.) of lorglumide, a selective CCK₁ receptor antagonist, attenuated the hCG-induced inhibition of gastric emptying in Ovx rats, whereas central administration via the i.c.v. route did not. hCG treatment of Ovx rats inhibits gastric emptying in a dose-dependent manner via a peripheral mechanism of CCK hypersecretion and activation of CCK₁ receptors.

Key Words

- ▶ human chorionic gonadotropin
- ▶ gastric emptying
- ▶ cholecystokinin
- ▶ ovariectomized rats

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Introduction

Disturbed gastrointestinal transit, leading to a delay in gastric emptying time, is one of the most common phenomenon in pregnant women (Chiloiro *et al.* 2001), and this effect is probably caused by changes in plasma concentrations of certain hormones, such as estrogen (Chen *et al.* 1995) and progesterone (Sheen-Chen *et al.* 2001), as concentrations of these hormones increase throughout pregnancy. Pregnant women with decreased gastrointestinal transit suffer from nausea, vomiting, or abdominal distension during the course of pregnancy, especially during the first trimester (Jarnfelt-Samsioe *et al.* 1983). The etiology of nausea and vomiting in pregnancy (NVP) is still unknown. Human chorionic gonadotropin (hCG), a hormone secreted by the placental trophoblast, has been proposed as the most likely cause of NVP, as hCG concentrations rise rapidly during the first trimester and peak at 10–12 weeks of gestation (Soules *et al.* 1980). Goodwin *et al.* (1994) reported that serum hCG concentrations are significantly increased in women with hyperemesis gravidarum compared with the control subjects of comparable gestational age. Furthermore, hCG concentrations correlate with the severity of vomiting (Goodwin *et al.* 1992). These findings suggest that hCG may play a role in the pathogenesis of NVP and gastrointestinal transit. However, there are no data on the relationship between hCG and gastrointestinal transit.

Cholecystokinin (CCK), a 33 amino acid peptide extracted from porcine intestine (Jorpes & Mutt 1966), inhibits gastric emptying in animals (Debas *et al.* 1975, Wu *et al.* 2008) and humans (Konturek *et al.* 1990) but has also been reported to stimulate colonic transit *in vivo* (Dinoso *et al.* 1973, Harvey & Read 1973). The biological actions of CCK are mediated by two types of receptors, CCK₁ and CCK₂ (Noble *et al.* 1999). CCK₁ receptors are present in both the CNS and various peripheral tissues, such as the gallbladder, pancreas, stomach, colon, and ileum, while the CCK₂ receptor is found predominantly in the CNS (Silvente-Poirot *et al.* 1993).

The aim of this study was to investigate the effects of hCG on gastric emptying and gastrointestinal transit in ovariectomized (Ovx) rats and to clarify the role of CCK and its receptors in regulating the effect of hCG on gastrointestinal transit. Furthermore, estrogen is important for maintaining normal uterine weight. Ovariectomy of rats without estrogen supplementation caused uterine atrophy. Decreased uterine weight is an important hallmark of success in ovariectomy surgery. Also, the influence of endogenous estrogen on gastrointestinal transit is relatively excluded. Hence, the change of uterine weight was observed in this study.

Materials and methods

Animals

Female Sprague Dawley rats weighing 250–300 g were housed in a temperature-controlled ($22 \pm 1^\circ\text{C}$) and light-controlled (0600–2000 h) environment and fed rat chow. Tap water was given *ad libitum*. Animal protocols were approved by the Institutional Animal Care and Use Committee of the National Yang-Ming University. All animals received care in compliance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals published by the National Science Council, Taiwan.

Surgery for insertion of i.c.v. cannulas

Surgery for insertion of i.c.v. cannulas was performed following the procedure described by Lin *et al.* (2003) with minor modification. Briefly, the rats were anesthetized by i.p. injection of phenobarbital (30 mg/kg) and placed in a stereotaxic frame. A stainless steel guide cannula was implanted according to the atlas of Paxinos and Watson, i.e. 0.8 mm posterior to bregma, 1.4 mm lateral to the midline, and 3.5 mm ventral to the dura. An AG-4 cannula (Eicom, Kyoto, Japan) was placed 4.5 mm below the scalp and secured in place with two anchor screws and dental acrylic (Tempo, GC Corporation, Tokyo, Japan). hCG was injected through the AG-4 cannula 1 week after insertion of the cannula.

Materials

The chemicals used in the study included recombinant hCG (Ovidrel; Merck Serono), EDTA, aprotinin, trifluoroacetic acid (TFA), and lorglumide sodium (all from Sigma Chemical Company), acetonitrile (Wako Chemical Industries, Ltd., Osaka, Japan), $\text{Na}_2^{51}\text{CrO}_4$ (DuPont NEN Research Products, Boston, MA, USA), and CCK EIA kit (Phoenix Pharmaceuticals, Belmont, CA, USA).

Experimental design

Effect of Ovx on gastric emptying Ten female rats were randomly divided into two groups of five each, group 1 as the control group (diestrus rats) and group 2, in which rats were bilaterally Ovx under i.p. phenobarbital (30 mg/kg) anesthesia. The rats were fasted overnight and

were then fed by gavage with a mixture of $\text{Na}_2^{51}\text{CrO}_4$ (0.5 $\mu\text{Ci}/\text{ml}$) and charcoal (10%) at a dose of 3 ml/kg. The rats were decapitated 15 min later, the uteri were weighed, and gastrointestinal transit was measured.

Effect of i.p. injection of hCG on gastric emptying and intestinal transit Forty female rats were randomly divided into four groups of ten each and were bilaterally Ovx under light ether anesthesia. Starting 2 weeks later, the test rats received a single daily i.p. injection of 50, 100, or 200 IU hCG for 2 days, while the control rats were injected with normal saline (1 ml/kg). After a 24-h fast, the rats received another i.p. injection of hCG (50, 100, or 200 IU) or normal saline, then, 45 min later, underwent gavage with a mixture of $\text{Na}_2^{51}\text{CrO}_4$ (0.5 $\mu\text{Ci}/\text{ml}$) and charcoal (10%) at a dose of 3 ml/kg, then were decapitated 15 min later, the uteri were weighed, and gastrointestinal transit was measured. Blood samples were collected for CCK EIA.

Effect of i.p. injection of lorglumide Twenty female rats were randomly divided into four groups and were bilaterally Ovx under light ether anesthesia. Starting 2 weeks later, groups 1 and 2 received an i.p. injection of saline, whereas groups 3 and 4 received 200 IU hCG daily for 2 days. After a 24-h fast, groups 1 and 3 received an i.p. injection of saline, while groups 2 and 4 received an i.p. injection of 10 mg/kg lorglumide. After 10 min, groups 1 and 2 received an i.p. injection of saline, whereas groups 3 and 4 received 200 IU hCG, then 45 min later underwent gavage with a mixture of $\text{Na}_2^{51}\text{CrO}_4$ (0.5 $\mu\text{Ci}/\text{ml}$) and charcoal (10%) at a dose of 3 ml/kg, then were decapitated 15 min later, and gastrointestinal transit was measured.

Effect of i.c.v. injection of lorglumide One week postovariectomy, 20 rats were divided into four groups of five each and an i.c.v. cannula was placed in the lateral ventricle. One week later, groups 3 and 4 were injected i.p. with 200 IU hCG once daily for 2 days, while groups 1 and 2 received saline. After an overnight fast, the animals were injected i.c.v. with 5 μl saline (groups 1 and 3) or 5 μl saline containing 5 nmol lorglumide (groups 2 and 4) (Lin *et al.* 2003). After 10 min, groups 1 and 2 received an i.p. injection of saline, whereas groups 3 and 4 received 200 IU hCG. Forty-five minutes later, the rats underwent gavage orally with a mixture of $\text{Na}_2^{51}\text{CrO}_4$ (0.5 $\mu\text{Ci}/\text{ml}$) and charcoal (10%) at a dose of 3 ml/kg and were decapitated 15 min later and gastrointestinal transit was measured.

Measurement of gastric emptying and gastrointestinal transit

Gastric emptying and gastrointestinal transit were measured as described by Doong *et al.* (1998). The rats were fed by gavage via a catheter (PE-205, inner diameter (ID) 1.67 mm, outer diameter (OD) 2.42 mm, Clay-Adam, Parsippany, NJ, USA) with physiological saline (3 ml/kg) containing $\text{Na}_2^{51}\text{CrO}_4$ (0.5 $\mu\text{Ci}/\text{ml}$) and 10% charcoal, which was stirred continuously before use. Additional air (0.5 ml) was used to flush the residual charcoal suspension in the catheter into the rat. Fifteen minutes later, the rats were decapitated and the stomach and attached small intestine were immediately exposed by laparotomy. After ligation of the esophagogastric, gastroduodenal, and ileocecal junctions, the whole stomach and small intestine were carefully removed and placed on a wooden board to observe the leading edge of the charcoal within the intestine. The small intestine was then divided into ten equal segments, and the radioactivity in the stomach and

Figure 1 Comparison of gastric emptying (top panel) and gastrointestinal transit (bottom panel) in diestrus and Ovx rats. ** $P < 0.01$ compared the diestrus group. Each column represents the mean $\pm$ s.e.m. ($n = 5$).

Figure 2

Effect of different doses of hCG on gastric emptying (top panel) and gastrointestinal transit (bottom panel) in Ovx rats. After an overnight fast, the rats were fed a mixture of charcoal (10%) and $\text{Na}_2^{51}\text{CrO}_4$ (0.5 $\mu\text{Ci/ml}$) by gavage, then were decapitated 15 min later, and gastric emptying and gastrointestinal transit were measured. * $P < 0.05$ and ** $P < 0.01$ compared to the control group. Each column represents the mean $\pm$ S.E.M. ($n = 10$).

each segment of small intestine was measured using an automatic gamma counter (1470 Wizard; Pharmacia). Gastric emptying was measured by determining the amount of labeled chromium contained in the small intestine 15 min after gavage expressed as a percentage of the amount given (Holzer 1985, Doong *et al.* 1998). Gastrointestinal transit was assessed at the same time by calculating the geometric center of distribution of the radioactivity within the ten segments (Miller *et al.* 1981, Chen *et al.* 1997). The geometric center was calculated by summation of the percentage of the radioactivity in each segment multiplied by the segment number.

Processing of plasma

After decapitation, blood samples were collected and mixed with EDTA (1 mg/ml blood) and aprotinin (500 KIU/ml blood), and plasma was immediately prepared by centrifugation at 1000 g for 30 min at 4 °C and used for measurement of plasma CCK concentrations.

The plasma samples were acidified with an equal volume of 1% TFA and then centrifuged at 6000 g for 20 min at 4 °C. A SEP-PAK C_{18} cartridge (Waters Associates, Milford, MA, USA) was equilibrated with 1 ml 60% acetonitrile in 1% TFA, followed by 3 $\times$ 3 ml 1% TFA, and then the supernatant from the treated plasma sample was applied. After washing with 2 $\times$ 3 ml 1% TFA, the peptide was eluted with 3 ml 60% acetonitrile in 1% TFA, dried down in a Speed Vac concentrator (Salvant Instruments, Farmingdale, NY, USA), the dried samples were stored at -70°C , and reconstituted with the appropriate assay buffer before CCK measurement (Jin *et al.* 1994, Wu *et al.* 2003). CCK was measured in duplicate using a commercial EIA kit (Phoenix Pharmaceuticals, Belmont, CA, USA); the inter- and intra-assay coefficients of variance were $< 10\%$, with a lower detection limit of 0.04 ng/ml.

Statistical analysis

All data are expressed as mean $\pm$ S.E.M. The treatment means were tested for homogeneity using one-way

Figure 3

Effect of hCG treatment on plasma CCK concentrations and correlation of gastric emptying with CCK concentrations in Ovx rats. Rats were treated as in Fig. 2, then plasma was collected and CCK concentrations were measured by EIA (top panel). Each column represents the mean $\pm$ S.E.M. ($n = 10$). * $P < 0.05$ and ** $P < 0.01$ compared to the control group. The bottom panel shows that gastric emptying rate was significantly negatively correlated with CCK concentrations in Ovx rats ($P = 0.01$, $r^2 = -0.5104$).

Figure 4

Effect of i.p. injection of lorglumide on the hCG-induced inhibition of gastric emptying (upper panel) and gastrointestinal transit (lower panel) in Ovx rats. Groups 1 and 2 received an i.p. injection of saline, whereas groups 3 and 4 received 200 IU hCG daily for 2 days. After a 24-h fast, groups 1 and 3 received an i.p. injection of saline, while groups 2 and 4 received an i.p. injection of 10 mg/kg lorglumide. Each column represents the mean $\pm$ S.E.M. ($n=10$). ** $P<0.01$ compared to the control group. ## $P<0.01$ compared to the group treated with hCG, but not lorglumide.

ANOVA and the significance of any difference between means tested using Duncan's multiple range tests. The Student's t -test was used for the statistical analysis of continuous variables. In all cases, the threshold for significance was considered as $P<0.05$.

Results

Changes in gastric emptying and intestinal transit in Ovx rats

Figure 1 shows a comparison of gastric emptying (top panel) and intestinal transit (bottom panel) in diestrus and Ovx rats. Gastric emptying was significantly increased in the Ovx rats ($P<0.01$), but there was no significant difference in intestinal transit. In addition, diestrus rats had a significant higher uterine weight than Ovx rats (data not shown; $P<0.01$).

Effect of hCG treatment on gastric emptying and intestinal transit in Ovx rats

As shown in Fig. 2, treatment of Ovx rats with 50, 100, or 200 IU hCG for 3 days resulted in significant inhibition of gastric emptying in a dose-dependent manner ($P<0.05$ or $P<0.01$), with no significant difference in intestinal transit.

Effect of hCG treatment on plasma CCK concentrations in Ovx rats

As shown in Fig. 3, treatment with 50, 100, or 200 IU hCG for 3 days resulted in a dose-dependent increase in plasma CCK concentration (top panel, $P<0.05$ or $P<0.01$). Furthermore, gastric emptying showed a significant negative correlation with CCK concentrations (bottom panel; $P=0.01$, $r^2=-0.5104$).

Figure 5

Lack of effect of i.c.v. injection of lorglumide on the hCG-induced inhibition of gastric emptying (upper panel) and gastrointestinal transit (lower panel) in Ovx rats. Groups 1 and 2 received saline, while groups 3 and 4 were injected i.p. with 200 IU hCG once daily for 2 days. After an overnight fast, the animals were injected i.c.v. with 5 μ l saline (groups 1 and 3) or 5 μ l saline containing 10 μ g/kg lorglumide (groups 2 and 4). Each column represents the mean $\pm$ S.E.M. ($n=5$). ** $P<0.01$ compared to the control group.

Effect of i.p. injection of lorglumide on the hCG-mediated inhibition of gastric emptying and intestinal transit in Ovx rats

Starting 2 weeks after Ovx, groups 1 and 2 received an i.p. injection of saline, whereas groups 3 and 4 received 200 IU hCG daily for 2 days. After a 24-h fast, groups 1 and 3 received an i.p. injection of saline, while groups 2 and 4 received an i.p. injection of 10 mg/kg lorglumide. After 10 min, groups 1 and 2 received an i.p. injection of saline, whereas groups 3 and 4 received 200 IU hCG and then gastrointestinal transit was measured. As shown in Fig. 4, lorglumide treatment significantly reduced ($P < 0.01$) the hCG-induced inhibition of gastric emptying (top panel) but had no effect on intestinal transit (bottom panel).

Lack of effect of i.c.v. injection of lorglumide on the hCG-mediated inhibition of gastric emptying and intestinal transit in Ovx rats

One week postovariectomy, 20 rats were divided into four groups and an i.c.v. cannula inserted into the lateral ventricle. One week later, groups 3 and 4 were injected i.p. with 200 IU hCG once daily for 2 days, while groups 1 and 2 received saline. After an overnight fast, the animals were injected i.c.v. with 5 μ l saline (groups 1 and 3) or 5 μ l saline containing 5 nmol of lorglumide (groups 2 and 4). After 10 min, groups 1 and 2 received an i.p. injection of saline, whereas groups 3 and 4 received 200 IU hCG, and then gastrointestinal transit was measured. As shown in Fig. 5, lorglumide had no significant effect on hCG-induced inhibition of gastric emptying (top panel) or intestinal transit (bottom panel).

Discussion

In this study, we showed that hCG treatment resulted in dose-dependent inhibition of gastric emptying in Ovx rats but had no effect on intestinal transit. Furthermore, hCG increased plasma CCK concentrations in a dose-dependent manner in Ovx rats, and gastric emptying showed a significantly negative correlation with CCK concentrations. This showed that, in Ovx rats, hCG inhibited gastric emptying, but not intestinal transit, through a mechanism involving increased secretion of CCK, a finding that has not been previously described. These results might explain the mechanism of the delay in gastric emptying time in pregnant women during the first trimester of pregnancy.

This study demonstrated that Ovx rats showed increased gastric emptying but not intestinal transit compared with diestrus rats. Estradiol (E_2) treatment inhibits gastric emptying in Ovx rats (Wu *et al.* 2002, 2008). This study also demonstrated that E_2 and a combination of E_2 and progesterone inhibit gastric emptying, but progesterone alone enhances gastric emptying in Ovx rats (Chen *et al.* 1995). Furthermore, in male rats, low-dose progesterone has been reported to increase gastric emptying, while high-dose progesterone inhibits it (Liu *et al.* 2002). In this study, increased gastric emptying was seen in Ovx rats, as secretion of E_2 and progesterone was blocked due to ovariectomy. Further studies are needed to clarify the contribution of hypo-secretion of E_2 and progesterone on enhanced gastric emptying in Ovx rats.

Pregnant women often complain of nausea, vomiting, and abdominal distension, especially during the first trimester (Jarnfelt-Samsioe *et al.* 1983). hCG is secreted by the placental trophoblast and its concentrations rise rapidly during the first trimester and peak between 10 and 14 weeks of gestation (Soules *et al.* 1980). This peak corresponds to the onset and severity of the symptoms in the first trimester in pregnant women. This study showed that hCG treatment inhibited gastric emptying in Ovx rats, suggesting that inhibition of gastric emptying by hCG may be the possible mechanism of NVP. Furthermore, the results of this study suggest that intestinal transit may not be affected by hCG in pregnant women. However, even though hCG was applied to mimic the first trimester in pregnant women, the present animal model does not completely mimic pregnant women, in whom E_2 and progesterone are secreted not only by the ovaries but also by the placenta. A further study is needed.

In addition to the corpus luteum rescue and progesterone production, hCG executes many extragonadal actions to initiate and maintain pregnancy, such as development of uterine and placenta, invasion of trophoblast modulation of maternal immune response, differentiation of fetal organs, etc. Study shows that there are three other variants of hCG, including hyperglycosylated hCG (hyp-hCG), β -subunit of hyperglycosylated hCG (hCG-free β), and pituitary hCG. Hyp-hCG plays a regulatory role in trophoblast invasion and placentation, hCG-free β plays a regulatory role in cancer malignancy and cellular transformation, and pituitary hCG plays a regulatory role in the menstrual cycle. hCG performs its gonadal actions through binding with the hCG/LH receptor on corpus luteal cells of the ovary. Besides, the hCG/LH receptor is also detected in several non-gonadal tissues and organs,

including the uterus, mammary gland, adrenal gland, oviduct, brain, etc. These extragonadal hCG/LH receptors make it possible for hCG to perform its extragonadal functions (Cole 2010, Banerjee & Fazleabas 2011). On the other hand, CCK is synthesized by enteroendocrine I-cells of the intestinal mucosa. Besides, some studies demonstrated that CCK is detectable in some pituitary and adrenomedullary cells. Also, CCK is synthesized in neurons of the brain and gut (Liddle 2000). According to the above description, it is possible that hCG could stimulate CCK release through binding with extragonadal hCG/LH receptors in the brain, small intestine, or neurons and then inhibited gastric emptying. However, further studies should be performed to prove this relationship.

CCK inhibits gastric emptying in both animals and humans (Chey *et al.* 1970, Jin *et al.* 1994), and this may explain why endogenous CCK decreases food intake in humans and animals (Gibbs *et al.* 1976, Ballinger & Clark 1994). CCK inhibits gastric emptying by relaxing the proximal part of the stomach and stimulating contraction of the pyloric sphincter (Chey *et al.* 1970, Fisher *et al.* 1973). CCK also inhibits gastric secretion in humans (Konturek *et al.* 1990). In this study, hCG treatment of Ovx rats resulted in a fact that the inhibition of gastric emptying induced by hCG might be associated with CCK hypersecretion and that inhibition of gastric emptying caused by hypersecretion of hCG and CCK may explain the gastric distension and NVP seen in women during the first trimester of pregnancy.

CCK inhibits gastric emptying by stimulating CCK receptors (Ballinger & Clark 1994, Varga & Scarpignato 1996). Two G-protein-coupled CCK receptors are involved, CCK₁ and CCK₂. CCK₁ receptors are abundant in peripheral organs (Silvente-Poirot *et al.* 1993), while the CCK₂ receptor is the major CCK receptor in the brain (Silvente-Poirot *et al.* 1993). Recently, the CCK₁ receptor was reported in numerous CNS regions (Moran *et al.* 1986, Mercer *et al.* 2000). To investigate the mechanism of hCG-induced inhibition of gastric emptying, the selective CCK₁ receptor antagonist lorglumide was injected into Ovx rats by either the i.p. or i.c.v. route and was found to attenuate the hCG-induced inhibition of gastric emptying when given i.p., but not when given i.c.v. These results suggest that hCG induces inhibition of gastric emptying in Ovx rats by a peripheral mechanism involving the activation of CCK₁ receptors, a previously unreported finding.

In conclusion, hCG treatment of Ovx rats inhibits gastric emptying in a dose-dependent manner via a

peripheral mechanism involving CCK hypersecretion and CCK₁ receptor activation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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