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• BASIC RESEARCH •

Effects of progesterone on gastric emptying and intestinal transit in male rats

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Abstract

AIM: **To study the dose-dependent of progesterone (P) effect and the interaction between the oxytocin (OT) and P on gastrointestinal motility.**

METHODS: **In order to monitor the gastric emptying and intestinal transit, the SD male rats were intubated via a catheter with normal saline (3ml/kg) containing $Na_2{}^{51}CrO_4$ (0.5μCi/ml) and 10% charcoal. OT was dissolved into normal saline and P was dissolved into 75% alcohol.**

RESULTS: **Low does of P (1mg/kg, i.p.) enhanced the gastric emptying (75±3%, *P*<0.05) and high dose of P (5mg/kg, i.p.) inhibit it (42±11.2%, *P*<0.01). P (1mg/kg) increased the intestinal transit (4.2±0.3, *P*<0.05) while the higher dose (10-20mg/kg) had no effect. OT (0.8mg/kg, i.p.) inhibited the gastric emptying (23.5± 9.8%, *P*<0.01). The inhibitory effects of P(20mg/kg) (32±9.7%, *P*<0.05) and OT (0.8mg/kg) on gastric emptying enhanced each other when the two chemicals were administrated simultaneously (17±9.4%, *P*<0.01).**

CONCLUSION: **Low dose of P increased GI motility while high dose of P decreased it. During the later period of pregnancy, elevated plasma level of OT may also participate in the gastrointestinal inhibition.**

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INTRODUCTION

Gastrointestinal motility is disturbed in pregnant women because of the changes of some hormone levels in the plasma, such as estrogen (E), progesterone (P), and other gastrointestinal hormones[1,2]. Steroids, especially P and E, participate the regulation of gastrointestinal motility[3-5] and are involved in the pathogenesis of some functional disorders in the gut[6]. We reported that administration of estrogen inhibited the rat gastric emptying and intestinal transit while P enhanced the gut motility[7]. The effects of E were testified by other scholars while the inhibitory effect of P was observed[8]. Oxytocin (OT) is also a hormone related to pregnancy, and its plasma concentration is higher during the late phase[9, 10]. It is reported that OT inhibited gastric motility and secretion[11 - 13]. We recently found that administration of OT inhibited the rat gastrointestinal motility through inducing the release of cholecystokinin (CCK) (unpublished data). Progesterone affects the OT effect on uterine smooth muscle[14], but it is unknown if P and OT interact with each other on the effect of gut motility. In this study, three experiments were conducted to investigate the dose-dependent effect of P and the interaction between P and OT on gastric emptying and intestinal transit in male rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 250-350g were housed at 22℃ and light-controlled environment (6a.m.-8p. m.) and fed with rat chow. Tap water was given *ad libitum*.

Experiment protocol

Experiment 1. The male rats were randomly divided into four groups (*n*=6each). They were fasted but with access to water for 24h before use. On the day of experiment, the four groups of animals were injected intraperitoneally (i. p.) with 0, 0.3, 1.0 and 3.0mg/kg of P, respectively 15min before gastric intubation of a non-nutrient liquid meal. Fifteen min after the administration of the liquid meal, the rats were decapitated and the gastric emptying and intestinal transit were measured.

Experiment 2. The procedure was identical to that in experiment 1, except the dose of *P* was 0, 5, 10 and 20mg/kg, respectively in the four groups of animals (*n*=7).

Experiment 3. Male rats were divided into four groups with 7 animals each and fasted for 24h before use. Fifteen min before gastric intubation of a non-nutrient liquid meal, the animals were injected i.p. with the normal saline and 75% alcohol (1ml/kg) in group 1, OT (0. 8mg/kg) and 75% alcohol (1ml/kg) in group 2, normal saline (1ml/kg) and P (20mg/kg) in group 3, and OT (0.8mg/kg) and *P* (20mg/kg) in group 4. OT was dissolved in the normal saline and *P* was dissolved in the alcohol (75%).

Measurement of gastric emptying and GI transit

Gastric emptying and GI transit were measured as described by Doong *et al*[15]. Rats were intubated via a catheter (PE-205, ID 1. 67mm, OD 2.42mm, Clay-Adam, Parsippany, NJ, USA) with normal saline (3mL/kg) containing $Na_2{}^{51}CrO_4$ (0.5μCi/mL) and 10% charcoal. The test meal was continuously stirred before intubation. Air (0.5mL) was used to flush the residual charcoal suspension in the catheter into the rats. Fifteen min later, the rats were decapitated and the stomach and attached small intestine 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 in the intestine. The small intestine was then divided into 10 equal segments and the radioactivity in the stomach and each segment of small intestine was measured in an automatic gamma counter (1470 Vizard, Pharmacia, Turku, Finland). Gastric emptying was measured by determining the amount of labeled chromium contained in the small intestine 15min after

intubation, expressed as a percentage of the amount given. Intestinal transit was assessed by calculating the geometric center of distribution of the radioactivity with the 10 segments by summation of the radioactivity in each segment multiplied by the segment number.

Statistical analysis

The data were expressed as the mean value ± S. E. M. The treatment means were tested for homogeneity using one-way analysis of variance, and the significance of any difference between means was tested using Duncan's multiple range test. A difference between two means was considered to be statistically significant when P was less than 0.05.

RESULTS

Low dose of P on gastric emptying and intestinal transit

Gastric emptying was enhanced slightly after P (0.3mg/kg) was injected i. p., and was elevated significantly by administration of P (1mg/kg) in the same way (P<0.05, n=6). P (3mg/kg) inhibited the gastric emptying, although the difference was not significant (Figure 1-1). Administration of P, 1mg/kg or 3mg/kg, increased the intestinal transit significantly (P<0.05, n=6), but the dose of 0.3mg/kg did not influence it (Figure 1-2).

High dose of P on gastric emptying and intestinal transit

Administration of P (5-20mg/kg, i.p.) dose dependently decreased the gastric emptying (Figure 2-1). In this experiment, only the lowest dose of P (5mg/kg, i.p.) significantly increased the intestinal transit, P with much higher dose (10 and 20mg/kg) did not influence the intestinal transit (Figure 2-2).

Interaction of OT and P on gastric emptying and intestinal transit

Compared with control, both OT (0.8mg/kg, i.p.) and P (20mg/kg i.p.) significantly inhibited the gastric emptying. When both OT and P were administrated, the gastric emptying was further inhibited (Figure 3). Administration of OT (0.8mg/kg) and/or P (20mg/kg) did not influence the intestinal transit.

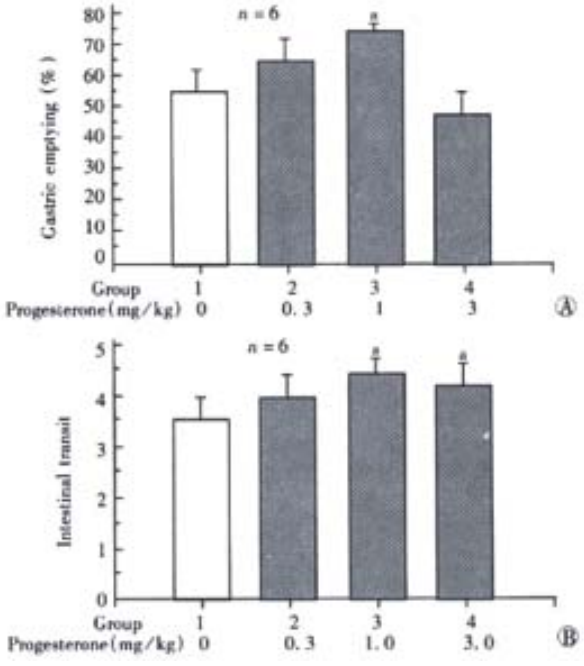

Figure 1 A:Effect of low dose of progesterone (i.p.) on gastric emptying in male rats. ^{a}P<0.05 *vs* group 1;B: Effect of low dose of progesterone (i. p.) on intestinal transit in male rats. ^{a}P<0.05 *vs* group 1

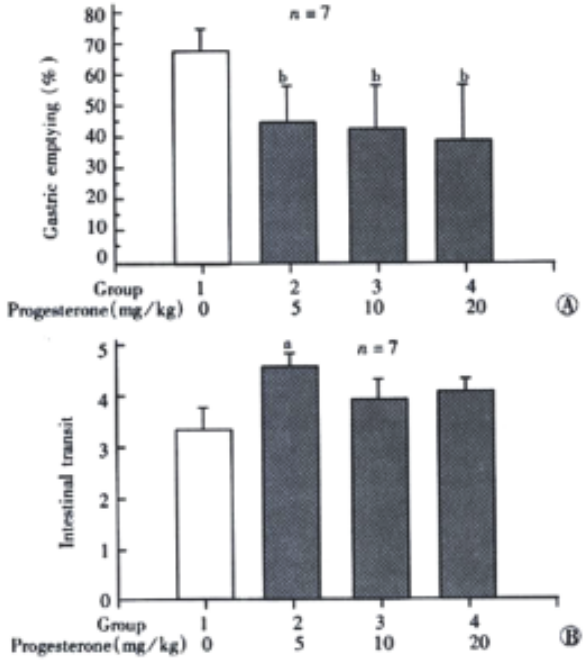

Figure 2 A:Effect of high dose of progesterone (i.p.) on gastric emptying in male rats. ^{b}P<0.01 *vs* group 1; B: Effect of high dose of progesterone (i.p.) on intestinal transit in male rats. ^{a}P<0.05 *vs* group 1

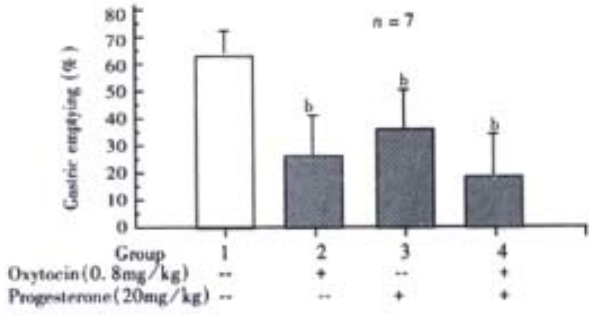

Figure 3 Interaction of progesterone and oxytocin (i.p.) on gastric emptying in male rats. ^{b}P<0.01 *vs* group 1

DISCUSSION

It is known that the liquid emptying slows as the calorie content increases. For nutrient liquid that stimulates intestinal feedback inhibition, there is an initial rapid emptying phase followed by a linear phase. In this study, we used non-nutrient liquid meal, and therefore the emptying rate was rapid, representing the initial rapid emptying of nutrient liquid. Because the volume in the initial rapid emptying contributes greatly to the nutrient liquid being half emptied, it is reasonable to assume that the changes of liquid emptying caused by the hormones from our study could influence the half-emptying time of nutrient liquid.

The result in this study indicated that the lower dose of P enhanced the gastrointestinal motility while the higher dose inhibited it. This data could explain why different effects of P on gastric emptying and intestinal transit were observed. Different routes of administration could make the chemicals absorbed at different velocity, thus making the plasma level of this hormone different. Besides the central nervous system, other organs such as the uterus also secrete OT into blood[16, 17]. In the pregnant women with or without pain, the OT concentration in plasma is much higher than that in the cerebrospinal fluid, so the higher level of OT during the late period of pregnancy may mainly come from the peripheral organs[18].

Although there is no direct evidence that there exists OT receptor in the gut, our data indicated that OT could inhibit the gastric emptying.

Disturbed gastrointestinal motility was observed in the pregnant women. In the early period, the high level of estrogen and progesterone may contribute to this pathophysiological phenomenon. During the late period, the OT level in plasma was elevated[9, 10]. In the present study, when administrated simultaneously, the inhibitory effects of P and OT on gastrointestinal were strengthened each other. Thus, OT may also participate in the inhibition of gastrointestinal motility during the late period of pregnancy.

In the uterus, The OT receptor is upregulated in the secretary phase during the oestrous cycle and downregulated during the early pregnancy[19-21]. Two levels of interaction between OT and steroid hormones were reported, the genomic and non - genomic mechanisms[22]. Estrogen induces the OT receptor (OTR) mRNA expression, and then increases the OTR density on the membrane of the uterus smooth muscle[22-35]. This effect of estrogen was also observed in the central nervous system[36]. The effect of P on OTR expression is controversial. It has been suggested that, through the nuclear receptors, P inhibits the OTR mRNA expression, and makes the target cell less sensitive to OT stimulation[25, 26, 37-43]. Giacalone also reported that, RU-486, a P receptor antagonist, increases the incidences of tachysystole, hypertonia and fetal heart rat abnormality[44]. However, Al-Matubsi reported that pretreatment with P enhanced the OTR mRNA expression in the ovine corpus luteum[45]. OT excites the uterus smooth muscle mainly through two mechanisms: binding the membrane receptor directly and increasing the secretion of prostaglandin (PG)[46-48]. P enhanced uterine PGF 2á secretion in response to OT in ovaritctomized sows[49].

Direct interaction, but not through the regulation of RNA expression, between P and OT was also reported. Grazzini *et al* found that P specially binded to the OTR and inhibited its ligand binding and signal functions[14, 50]. Burger also reported that P inhibited the calcium signaling evoked by ligand stimulation of G protein-coupled receptor. This kind of P effect was fast, reversible and was not prevented by cycloheximide, indicating a non-genomic nature[51].

In this experiment, administration of P (20mg/kg) and OT (0.8mg/kg) in combination inhibited the gastric emptying, and the gastric motility was further attenuated. Because this effect was found within 30min after the chemical treatment, it is clear that the nature of the potentiation between P and OT is non - genomic.

In conclusion, the data indicated that high dose of P inhibited the GI motility while low dose of P enhanced it. During pregnancy, especially during the later period, high levels of P and OT enhance each other to disturb the gastrointestinal motility.

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