BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1994 Mar; 15 (2): 129—132

Effects of endothelin-1 on isolated uterine horns in estrogen-primed and pregnant mice[1]

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ABSTRACT Mouse uterine horns from 4 states (estrogen-primed and early-, mid-, and late-pregnancy) were used to study the effect of endothelin-1 (ET) *vs* carboprost (Car) and oxytocin (Oxy). In K^+-Krebs (KCl 40 mmol $\cdot L^{-1}$) solution, ET (1—300 nmol $\cdot L^{-1}$), Car (0.002—20 μmol $\cdot L^{-1}$), and Oxy (0.6—60 nmol $\cdot L^{-1}$) evoked concentration-dependent increases in tension of the uterine horns from 4 different states. E_{max} for ET were 1.12 ± 0.26, 1.27 ± 0.18, and 1.49 ± 0.13 g in early-, mid-, and late-pregnancies, respectively. E_{max} for Car in mid- was twice that in late-pregnancy, whereas E_{max} for Oxy in late- was thrice that in mid-pregnancy. EC_{50} for ET were 9.6, 5.8, and 6.3 nmol $\cdot L^{-1}$ in early-, mid-, and late-pregnancies, respectively, and were only 2 % to 7 % of that for Car and 3—15 times of that for Oxy in various gravid stages. The results suggest that the contractile activity of pregnant mouse uterus to ET is more potent than that of Car while slightly weaker than that of Oxy.

KEY WORDS endothelins; oxytocin; carboprost; uterine contraction; animal pregnancy; drug dose-response relationship

Endothelin-1 (ET) has a potent contractile activity on blood vessels[1-3], trachea, ileum, urinary bladder, vas deferens, and uterus[4-8]. In isolated rat uterine horns, ET enhances the rhythmicity and the magnitude of contraction[6]. In estrogen-primed mice, ET provokes the contraction of depolarized uterine horns[7]. This study was designed to compare the contractile activity of ET on mouse uterus at different stages of pregnancy with those of carboprost (Car, 1, 5-methylprostaglandin $F_{2\alpha}$) and oxytocin (Oxy).

Received 1993-01-20 Accepted 1993-11-18
[1] Project supported by the National Natural Science Foundation of China, № 38970831.

MATERIALS AND METHODS

Thirty-six pregnant and 12 estrogen-primed Kunming ♀ mice, weighing 28.5 ± *s* 0.5 g (6—8 wk) before mating and estrogen treatment were used. Two ♀ mice were caged overnight with 1 ♂ (2:1). Positive insemination was determined by the presence of copulation plugs the next morning. The day on which the copulation plugs were found was taken as d 1 of pregnancy. Experiments were performed on uterine horns isolated from early (d 6)-, mid (d 12—13)-, and late (d 18—19)- pregnant, and estrogen-primed mice (estradiol benzoate (1 mg $\cdot kg^{-1} \cdot d^{-1}$) was injected ip 48 h and 24 h before kill).

Preparation Mice were killed by cervical dislocation. Uterine segment (1 cm) was suspended in a 5-ml glass chamber filled with Krebs solution at 37 ± 0.5 ℃ and gassed with 95 % O_2 + 5 % CO_2. Isometric tension was continuously recorded. Uterine segments were allowed to equilibrate at a resting tension of 1 g for 1 h. For early-, mid-, and late-pregnant and a part of estrogen-primed uteri, Krebs solution was then replaced by K^+-Krebs solution (KCl 40 mmol $\cdot L^{-1}$, NaCl 82.6 mmol $\cdot L^{-1}$), in which the tension of uterine segment was first enhanced sharply and then relaxed gradually. Meantime the rhythmic contraction disappeared. The tension reached a plateau in 20—30 min and then readjusted to 1 g.

Drugs Endothelin-1 (Peninsula Laboratories Inc, Belmont CA 94002, USA), carboprost (Shanghai

Wu-Zhou Pharmaceutic Factory), oxytocin (injection, 5 IU · ml^{-1}, Shanghai Tian-feng Pharmaceutic Factory) and estradiol benzoate (injection, 2 mg ·ml^{-1}, Shanghai № 9 Pharmaceutic Factory). ET, Car, and Oxy were dissolved in distilled water, and estradiol benzoate was dissolved in olive oil.

Drug responses ET, Car or Oxy was added in a cumulative fashion. The contractile response to each concentration was expressed as the increment in tension (g). The cumulative concentration-response curves (CCRC) for the drugs in early-, mid-, and late-pregnant, and estrogen-primed uteri were obtained. Only 1 CCRC was constructed from each preparation.

Statistics Data were expressed as $\bar{x}\pm s$. Differences in E_{max} for ET, Car, and Oxy between stages of pregnancy or drugs were assessed by t-test. EC_{50} and 95 % confidence limits for each CCRC were calculated by the method of weighted regression line with a computer program. Differences between two EC_{50} were determined by unoverlaping of the two 95 % and 99 % confidence limits.

RESULTS

Effects of ET and Car on estrogen-primed mouse uterus In Krebs solution, the uterine horn manifested a spontaneous rhythmic contraction. Both ET (1 — 300 nmol·L^{-1}) and Car (0.002 — 20 μmol·L^{-1}) induced concentration-related contractions with an increase in both magnitude and frequency of rhythmic contraction and in the tension. When the concentrations of ET and Car reached 300 nmol·L^{-1} and 20 μmol·L^{-1}, respectively, a tonic contraction ensued.

In K$^+$-Krebs solution, the rhythmic contractions of the depolarized myometrium were completely inhibited, whence ET 1—300 nmol ·L^{-1} or Car 0.002 — 20 μmol·L^{-1} merely induced an increase in tension of the muscle in a concentration-dependent fashion. The tension induced by different concentrations of ET and Car in both Krebs and K$^+$-Krebs solutions were shown in Fig 1. The maximal increase in tension (E_{max}) induced by either ET or Car was higher in Krebs solution than that in K$^+$-Krebs solution ($P<0.01$). Their EC_{50} values were not significantly different (Tab 1).

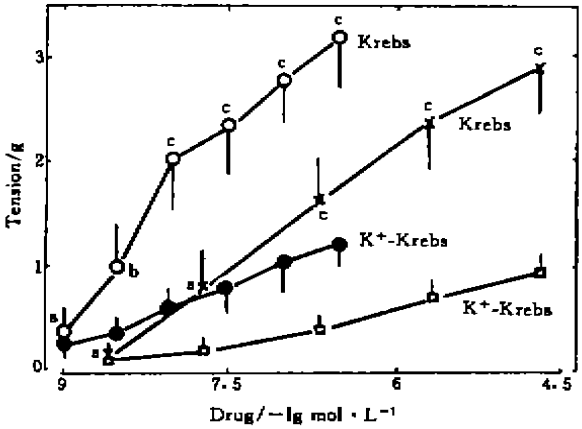

Fig 1. Tension of uterine horns in estrogen-primed mice caused by endothelin-1 (○, ●) and carboprost (×, □) in either Krebs or K$^+$-Krebs solutions. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* K$^+$-Krebs solution.

Tab 1. E_{max} and EC_{50} of increase in tension in estrogen-primed mice uteri caused by ET and Car in Krebs and K$^+$-Krebs solution. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^cP<0.01$ *vs* Krebs solution.

Drug	Solution	E_{max}/g	EC_{50} (95% confidence limits)/nmol·L^{-1}	
ET	Krebs	3.2±0.5	8.2	(4.1—16.6)
	K$^+$-Krebs	1.21±0.19^c	9.3^a	(5.0—17.4)
Car	Krebs	3.0±0.5	157	(45—541)
	K$^+$-Krebs	0.97±0.16^c	205^a	(88—479)

Effects of ET, Car, and Oxy on depolarized myometrium in pregnant mice In K$^+$-Krebs solution, ET 1 — 300 nmol·L^{-1}, Car 0.002 — 20 μmol·L^{-1}, and Oxy 0.6 — 60 nmol ·L^{-1} evoked concentration-dependent increases in tension of the muscle at all 3 pregnant stages (Fig 2). The maximal contractile responses to ET in early-, mid-, and late-pregnant uteri were not significantly different, except that it was higher in late- than in early-pregnant uteri (Tab 2, Fig 2). The E_{max} for Car 20 μmol·L^{-1} was higher in early- and mid-

pregnant stages than that in late-pregnant stage (Tab 2, Fig 2). For Oxy, the order of relative heights of E_{max} was late- > early- > mid-pregnant stage (Tab 2, Fig 2). In late-pregnant stage, the maximal increase in tension caused by ET was much more than that caused by Car. In mid-pregnant uteri, E_{max} for ET was higher than that for Oxy ($P < 0.01$). EC_{50} values for ET were not significantly different among various pregnant stages. EC_{50} for ET were only 7 % and 2 % of those for Car in early - and mid - pregnant uteri, respectively. EC_{50} ratio of ET to Oxy were 7:1 in early-, 15:1 in mid-, and 3:1 in late-pregnant mice (Tab 2).

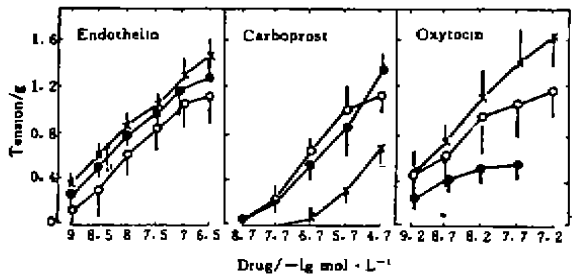

Fig 2. **Contraction of uterine horns in early- (○), mid- (●), and late - pregnant (×) mice induced by endothelin-1, carboprost, and oxytocin in K^+-Krebs solution.** $n = 6$, $\bar{x} \pm s$.

DISCUSSION

The present study showed that in Krebs solution ET mainly enhanced the magnitude and frequency of the rhythmic contraction at lower concentrations and raised the tension at higher concentrations. These were similar to its effects on isolated rat horns[6], but different from those on human uterine muscle (longitudinal and circular) in which ET 100 nmol · L^{-1} mainly increased the frequency of contraction[8]. The results indicated that ET had contractile action not only on rat and human uterine smooth muscle, but also on mouse uterus, and that its contraction enhancement activity in various species was different.

Tab 2. **E_{max} and EC_{50} of increase in tension in early-, mid-, and late-pregnant mice uteri caused by ET, Car, and Oxy in K^+-Krebs solution.** $n = 6$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* early-pregnancy; $^dP > 0.05$, $^eP < 0.01$ *vs* mid-pregnancy; $^gP > 0.05$, $^hP < 0.05$, $^iP < 0.01$ *vs* ET.

Drug	Stage of pregnancy	E_{max}/g	EC_{50} (95% confidence limits)/nmol · L^{-1}	
ET	early	1.12 ± 0.26	9.6	(4.2—21.6)
	mid	1.27 ± 0.18^a	5.8^a	(3.5—9.4)
	late	1.49 ± 0.13^{bd}	6.3^{ad}	(3.0—13.0)
Car	early	1.16 ± 0.19	138	(72—266)
	mid	1.36 ± 0.18^a	338	(109—1052)
	late	0.68 ± 0.12^{ei}		
Oxy	early	1.18 ± 0.22	1.4^h	(0.6—3.6)
	mid	0.53 ± 0.10^e	0.38	(0.23—0.63)
	late	1.7 ± 0.3^{bf}	2.4^g	(1.2—4.6)

We found that the sensitivity of myometrium to ET exhibited no significant different among early-, mid-, and late-pregnancy, and that the maximal response to ET was higher in late- than in early-pregnancy. This is different from either Car or Oxy. The threshold concentration of Car was 10 — 100 times higher in late-than in early- or mid-pregnancy (Fig 2), in accordance with that iv infusion of prostaglandin E_1 and E_2 on the motility of the pregnant human uterus[9]. E_{max} for Car in mid- was twice that in late-pregnancy, and for Oxy in late- was thrice that in mid-pregnancy. The maximal contractile response to ET was 2.2 times of that to Car in late-pregnancy, and 2.4 times of that to Oxy in mid-pregnancy.

We also found that EC_{50} of ET-induced contractile effect on uterine muscle in various pregnant stages were only 2 % and 7 % of those of Car whereas 3 — 15 times higher than those of Oxy. This indicated that the contractile response of uterine horn to ET was much more sensitive than that to Car and slightly less sensitive than that to Oxy.

It was reported that during pregnancy, estrogen concentration in rat ovarian venous plasma was generally low on d 1 to d 13 except on d 3 and d 4, rose gradually from d 13 to d 20, and thereafter rose sharply until parturition[11], and that progestin level increased from d 1 to d 14, then declined by the time of parturition[11]. In other words, there were low estrogen and high progesterone level on d 6, low estrogen and highest progesterone level on d 13, and high estrogen and low progesterone level on d 19. Maggi *et al* reported that estrogen dose-dependently increased the density of endothelin receptors in the rabbit uterus with an ED_{50} of 0.7 $\mu g \cdot kg^{-1}$ for 4 d and that the sequential administration of 17β-estradiol and progesterone (5 $mg \cdot kg^{-1}$ for 4 d) completely reversed the estrogen effect[12]. We speculated that the increase in estrogen secretion near term coupled with the declining progesterone levels led to increment in the density of endothelin receptors. This was responsible for the observation that the maximal contractile response to ET was the strongest in late-pregnancy. Whether the use of ET is applicable to the abortion in early pregnancy or to the induction of labor in mid - and late - pregnancy awaits further investigation.

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内皮素-1对雌激素处理及妊娠小鼠子宫

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A **摘要** ET，Car 和 Oxy 使在高钾液中的
处理和早、中、晚孕的小鼠子宫产生
性张力增加。ET 对晚孕子宫的 E_{max}
的1.33倍，Car 对中孕子宫的 E_{max} 为晚
倍，Oxy 对晚孕子宫的 E_{max} 是中孕者的
ET 对不同孕期子宫的 EC_{50} 无显著
Car 的2 % — 7 %，为 Oxy 的3—15倍
ET 对小鼠子宫的兴奋作用不同于 Car

关键词 内皮素；缩宫素；卡波前列腺
收缩；妊娠动物；药物剂量反应关系