Peripheral excitatory effects of two enkephalinase inhibitors, acetorphan and thiorphan, and an enkephalin analogue, [D-Ala²-Met⁵]-enkephalinamide, on uterine motility in periparturient rats *in vivo* and *in vitro*

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The effects of two enkephalinase inhibitors, acetorphan and thiorphan, and the enkephalin analogue [D-Ala²-Met⁵]-enkephalinamide (DAMEA), on spontaneous uterine contractions were studied at day 21 of pregnancy in rats following treatment *in vivo* or *in vitro*. Acetorphan (10 mg kg⁻¹) and thiorphan (1 mg kg⁻¹), immediately after their i.v. administration, increased the duration of spontaneous contractions 3.4- and 4.6-fold, respectively, but did not modify the maximum amplitude. Similarly, thiorphan (40 µmol l⁻¹) increased the duration of contractions when administered *in vitro*. Thiorphan was ineffective during the first 30 min when given into the cerebral ventricles (50 µg per rat). These results suggest that the enkephalinase inhibitors are acting via a peripheral opioid pathway; and this conclusion is supported by the observation that thiorphan potentiated the stimulatory effect of a submaximal dose of DAMEA administered *in vitro*. The excitatory effects of DAMEA and the enkephalinase inhibitors were blocked by naloxone. This antagonistic effect of naloxone on uterine motility in the periparturient rat uterus, induced by either acetorphan and thiorphan or DAMEA, seems to be regulated by peripheral opiate receptors. Naloxone (10 mg kg⁻¹ s.c.) increased both the amplitude and duration of uterine motility *in vivo*; however, naloxone (26 µmol l⁻¹ and 52 µmol l⁻¹) produced a paradoxical dose-dependent biphasic effect *in vitro*.

Introduction

Opioid peptides are involved in maternal adaptation to pregnancy and in uterine motility. In pregnant women, plasma concentrations of β -endorphin rise throughout pregnancy (Genazzani *et al.*, 1981), and reach peak values during labour (Facchinetti *et al.*, 1982; Genazzani *et al.*, 1985). This increase in opioid peptide concentrations during labour correlates in a linear fashion with the number and density of uterine contractions (Facchinetti *et al.*, 1982) and with response to the painful stimuli typical of this condition (Genazzani *et al.*, 1985). Furthermore, met-enkephalin concentrations increase during pregnancy and delivery in several brain regions of pregnant rats (Petraglia *et al.*, 1985) and in the human placenta during labour (Sastri *et al.*, 1980). Similarly, opiate receptors and enkephalinase, an enkephalin-degrading-enzyme at the Gly³-Phe⁴ bond (Malfroy *et al.*, 1978) are present in the pregnant rat uterus (Baraldi *et al.*, 1985; Ottelecz *et al.*, 1991) and in the human placenta (Valette *et al.*, 1980; Johnson *et al.*, 1984). These data suggest that met-enkephalin could be involved not only in the modulation of analgesia during pregnancy but also

in the regulation of uterine motility. However, little information is available concerning the action of enkephalin on the uterine muscle in the last stage of pregnancy. The present study was therefore undertaken to investigate the response of the rat myometrium to met-enkephalin on day 21 of pregnancy and the possible involvement of enkephalinase in this response, using a long lasting enkephalin analogue [D-Ala²-Met⁵]-enkephalinamide (DAMEA) and two enkephalinase inhibitors, acetorphan and thiorphan. Acetorphan, a lipophilic derivative of thiorphan enters into the brain, where it generates thiorphan (Roques *et al.*, 1980, 1981). When administered in low parental doses to rats, it inhibits the cerebral enkephalinase, producing more effects than does intracerebral thiorphan (Lecomte *et al.*, 1986). Thiorphan penetrates poorly into the brain and elicits mainly peripheral effects. Its nanomolar potency *in vitro* contrasts with the large parental doses required to inhibit the rat and mouse cerebral enzyme (Roques *et al.*, 1980; Chipkin *et al.*, 1982; Hachisu *et al.*, 1985). In addition to their analgesic properties, these inhibitors partially protect the endogenous met-enkephalin release from brain slices and from being extensively hydrolysed by enkephalinase (Patey *et al.*, 1981; De La Baume *et al.*, 1983) and increase the half-life of [³H]enkephalin (Chaillet *et al.*, 1983). Central and peripheral effects of these inhibitors were assessed by comparing the stimulatory actions of i.v. acetorphan and thiorphan, or thiorphan given i.v., i.c.v.

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or *in vitro*. The possible involvement of enkephalinase in the modulation of uterine motility was studied by adding DAMEA to the isolated tissue and attempting to protect this peptide with thiorphan. Naloxone, an opiate receptor antagonist, was used to block the effects induced by both inhibitors and DAMEA.

Materials and Methods

Animals

Adult Wistar rats (Charles River Labs, St Aubin-les-Elbeuf) were kept in a lighting schedule of 12 h light:12 h dark at $23 \pm 1^\circ\text{C}$ with free access to food (U.A.R.103, Villemoisson sur Orge) and water. Females (250–350 g body mass) were caged with males overnight and the vaginal smear examined for the presence of spermatozoa. The day on which spermatozoa were found in the smear was designated day 1 of pregnancy and animals were used at day 21. The average duration of gestation in the breeding colony was 22 days.

Preparation *in vivo*

Each animal was anaesthetized with sodium pentobarbital (45 mg kg^{-1} body mass, i.p.; Abbott Laboratories, St Remy sur Avre) and the trachea was cannulated to assist respiration. The abdomen was opened using a midline incision and the medial part of one uterine horn was linked to an isometric strain gauge connected to an ink recording polygraph. The uterus was secured to the strain gauge by a cotton thread passed between the myometrium and the blood vessels of the mesometrial membrane. The cervical end of the part of the uterus that was used for recording was secured to a vertical fixed metal rod to maintain isometric conditions. A standard tension of 1 g was applied to each uterine horn and care was taken to maintain irrigation by dripping Tyrode's solution (pH 7.4; 36°C) down the outside of the horn. This arrangement records isometric contractions primarily of the longitudinal muscle layer. The system was allowed to stabilize for 60 min before recordings were taken for 30 min to provide a control value for comparison with treatments. The vehicles in which the active agents were dissolved were administered by the appropriate route and in the appropriate volume at the start of the 30 min control period. Animals were then treated with the active agents and recordings taken for 30–60 min.

Isolated preparation *in vitro*

Each animal was killed with an overdose of pentobarbital and the uterine horn was split longitudinally. A $40 \text{ mm} \times 5 \text{ mm}$ uterine strip was removed, as described by Singh and Sharma (1984). The endometrium was removed by gentle but thorough scraping. The uterine strip was then washed several times with Krebs' solution (pH 7.4) at 4°C and mounted in a tissue chamber filled with 10 ml of Krebs' solution maintained at 36°C and gassed with 95% O_2 :5% CO_2 . Spontaneous isometric contractions (0.25 g resting tension) were recorded with a Gilson transducer (ICT 2H, Villiers-le-Bel) after

equilibration for about 60 min. A 15 min recording period with sterile saline was used as a control for subsequent 15 min exposures to active agents. In the bathing medium, the strip was washed between the addition of different drugs for at least 15 min and allowed to return to a stable baseline.

Treatment regimens

Acetorphan and thiorphan, synthesized as described by Roques *et al.* (1980, 1981), were obtained from Bioprojet Labs (Paris). Naloxone and [D-Ala²-Met⁵]-enkephalinamide (DAMEA) were purchased from Sigma Chemical Company (St Louis, MO). Thiorphan, naloxone and DAMEA were dissolved in sterile saline and the pH was adjusted, when necessary, to 7.4. Acetorphan was dissolved in a vehicle composed of 10% ethanol, 10% Cremophor (Bioprojet Labs), and 80% water, pH 7.4.

Experiments in vivo. Thiorphan was given i.v. at 0.15, 1 and 5 mg kg^{-1} body mass in 0.3 ml or i.c.v. at $50 \mu\text{g}$ in 20 μl . Acetorphan was given i.v. at 5 or 10 mg kg^{-1} in 0.3 ml. Naloxone was used to block the effects of the enkephalinase inhibitors. Naloxone was given s.c. at 5 or 10 mg kg^{-1} body mass in 0.3 ml, 10 min before i.v. thiorphan or acetorphan. This route and these doses of naloxone block the analgesic effects of acetorphan and thiorphan i.v. in rat and mice nociceptive tests. (Chaillet *et al.*, 1983; Costentin *et al.*, 1986; Lecomte *et al.*, 1986; Hachisu *et al.*, 1985).

Experiments in vitro. The met-enkephalin analogue, DAMEA, was used to reveal the presence of enkephalinase in the rat uterus. DAMEA was used at 3.7, 7.5 and $14.8 \mu\text{mol l}^{-1}$. Thiorphan at 10, 20 and $40 \mu\text{mol l}^{-1}$, was used alone or in combination with DAMEA ($13 \mu\text{mol l}^{-1}$). Naloxone at 13, 26 and $52 \mu\text{mol l}^{-1}$ was used alone or in combination with thiorphan ($40 \mu\text{mol l}^{-1}$) plus DAMEA ($13 \mu\text{mol l}^{-1}$).

Measurement of the spontaneous uterine contractions

Variations in myometrial mechanical activity were evaluated in terms of the duration and amplitude of contractions. The maximal amplitude (mm) of each contraction was measured and the duration (s) was taken as the interval between successive relaxations. The amplitude and duration of each contraction during the control period were calculated. Groups of 6–13 animals were assigned to each treatment *in vivo*, and each treatment *in vitro* was performed on at least 6–7 separate preparations. Each recording of the treatment *in vivo* lasted 30–60 min. This time was divided into 10 min periods. The means $\pm$ SEM of the amplitude and duration of contractions of each group of treated animals were calculated at 10 min periods during 30–60 min of recording. In the recording *in vitro*, these parameters were calculated during separate 15 min experiments.

Statistical analysis

Data for each group of 6–13 rats were analysed by analysis of variance and expressed as means $\pm$ SEM and are represented

in Tables 1 and 2. Data from the treated group mean and its own control were examined by Student's *t* test and differences were considered to be significant if $P < 0.05$.

Results

Experiments in vivo

Effect of naloxone. Naloxone, 5 mg kg^{-1} body mass (s.c.), did not induce any significant change in the amplitude or duration of uterine contractions over 30 min (Table 1). Naloxone, 10 mg kg^{-1} body mass, induced an immediate, significant increase (about 25%) in the amplitude of contractions, which was maintained over 60 min and a significant increase (40–50%) in the duration of contractions from 10–50 min after administration.

Effects of acetorphan alone or in combination with naloxone. Acetorphan, 5 mg kg^{-1} body mass (i.v.), had no significant effect on the amplitude or duration of uterine contractions over 30 min. The higher dose (10 mg kg^{-1} body mass) had no effect on amplitude but induced a significant increase (344%) in the duration of contractions during the first 10 min after administration. The duration of contractions had returned to pretreatment values by the 20–30 min sampling period. This effect was not seen when 5 mg naloxone kg^{-1} body mass was given 10 min before acetorphan. The vehicle in which acetorphan was dissolved had no effect on the amplitude or duration of uterine contractions over 30 min (data not shown).

Effects of thiorphan alone or in combination with naloxone. Intravenous injection of thiorphan, 0.15 and 1 mg kg^{-1} body mass, had no significant effect on the amplitude of uterine contractions but 5 mg thiorphan kg^{-1} induced a significant increase (about 30%) in the amplitude of contractions for the first 30 min of the 60 min observation period. All doses of thiorphan significantly increased the duration of uterine contractions; 5 mg thiorphan kg^{-1} produced an immediate tetanic contraction which lasted the entire 60 min observation period. Pretreatment with 10 mg naloxone kg^{-1} reduced the uterotonic effect of 1 mg thiorphan kg^{-1} . Pretreatment with 5 mg naloxone kg^{-1} was ineffective (data not shown).

Thiorphan, $50 \mu\text{g}$ per rat i.c.v., induced a significant increase (25–35%) in the amplitude of uterine contractions from 20 min after administration to the end of the 60 min observation period and a significant increase (33–60%) in the duration of contractions from 30 to 60 min.

Experiments in vitro

Effects of thiorphan, DAMEA and naloxone. Thiorphan, $10 \mu\text{mol l}^{-1}$, had no significant effect on the amplitude of uterine contractions during the 15 min observation, whereas 20 and $40 \mu\text{mol l}^{-1}$ significantly increased the amplitude of uterine contractions by 33 and 47%, respectively (Table 2). The highest concentration, $40 \mu\text{mol l}^{-1}$, also significantly increased the duration of contractions (262%).

DAMEA at concentrations of 3.7 , 7.4 and $14.8 \mu\text{mol l}^{-1}$ had no significant effect on the amplitude of contractions but the two higher concentrations, 7.4 and $14.8 \mu\text{mol l}^{-1}$, signifi-

cantly increased the duration of contractions by 73 and 220%, respectively.

Naloxone, $13 \mu\text{mol l}^{-1}$, had no effect on the amplitude or duration of uterine contractions, but naloxone, $26 \mu\text{mol l}^{-1}$, significantly increased both the amplitude (44%) and duration (53%) of contractions, whereas $52 \mu\text{mol}$ naloxone l^{-1} significantly decreased both the amplitude (56%) and duration (47%) of contractions compared with the pretreatment period.

Treatment with the met-enkephalin agonist, DAMEA, at the lowest concentration studied, $3.7 \mu\text{mol l}^{-1}$, which had no significant effect on the duration of uterine contractions when given alone, augmented the increase in the duration of uterine contractions seen with the enkephalinase inhibitor thiorphan at 10 , 20 and $40 \mu\text{mol l}^{-1}$, but did not alter the effect of thiorphan on the amplitude of contractions. DAMEA was added to the bathing medium 3 min after the addition of thiorphan.

The addition of the enkephalin antagonist naloxone, $26 \mu\text{mol l}^{-1}$, 5 min before the enkephalinase inhibitor thiorphan at the highest dose studied, $40 \mu\text{mol l}^{-1}$, inhibited the increase in the duration of contractions seen with thiorphan alone. Treatment with naloxone also inhibited the increase in the duration of contractions seen with the combination of thiorphan and DAMEA, although the highest concentration of naloxone, $52 \mu\text{mol l}^{-1}$, was required to exert this inhibitory effect and this dose of naloxone was inhibitory when given alone.

Discussion

It has been established that the control of myometrial activity during pregnancy is largely humoral and depends on intrinsic myogenic factors. Thus, some components are designed to keep the myometrium at rest and others to stimulate motility (Fuchs and Fuchs, 1984). Studies of the actions of opioids on the regulation of myometrial motility have been contradictory. Some investigators have shown that morphine inhibits the contractions of nonpregnant rat and mouse uteri (Widy-Tyszkiewicz *et al.*, 1978; Contreras *et al.*, 1982; Acevedo and Contreras, 1984). Others found that this opioid had no effect on rat uteri at parturition (Yaksh *et al.*, 1979) and β -endorphin caused only a small contraction of the postpartum rat uterus (Leng *et al.*, 1985). The results reported here present the effects of enkephalinase inhibitors and a met-enkephalin analogue upon spontaneous uterine mechanical activity of Wistar rats at day 21 of pregnancy (one day before parturition). Met-enkephalin and enkephalinase inhibitors, that is, acetorphan and thiorphan, stimulated the duration and, to a lesser extent, the amplitude of spontaneous uterine contractions. This result is in accordance with the action of enkephalin upon contractions of rat uteri at metoestrus (Ohia and Laniyonu, 1989). When enkephalinase inhibitor was given i.c.v. to the *in vivo* preparation, its effects were delayed compared with the effects following i.v. administration and its potentiation of the effects of DAMEA in the *in vitro* preparation. This finding suggests that the inhibitor was acting only when it had reached the periphery via the enkephalinase system. The pregnant rat uterus is known to contain higher concentrations of enkephalinase than does the nonpregnant uterus (Ottlecz *et al.*, 1991). However, this high concentration of enkephalinase

Table 1. Effects of acetorphan, thiorphan or naloxone administered alone and naloxone combined with acetorphan or thiorphan on maximal amplitude (A; mm) or duration (D; s) on spontaneous uterine contractions in periparturient rats *in vivo*

Agent used	Number of experiments	Control		Treatment					
		- 30-0 min		0-10 min	10-20 min	20-30 min	30-40 min	40-50 min	50-60 min
Acetorphan 5 mg kg ⁻¹ (i.v.)	10	A	29.0 ± 2.0	29.4 ± 3.5	28.2 ± 2.6	29.0 ± 0.9			
		D	54.0 ± 7.8	75.0 ± 19.0	65.4 ± 6.4	62.0 ± 8.0			
Acetorphan 10 mg kg ⁻¹ (i.v.)	13	A	30.0 ± 3.0	31.5 ± 2.8	30.2 ± 1.5	28.6 ± 3.5			
		D	61.9 ± 14.0	271.0 ± 74.0**	94.1 ± 24.0	68.8 ± 15.0			
Naloxone 5 mg kg ⁻¹ (s.c.)	8	A	22.0 ± 3.8	22.7 ± 4.8	24.0 ± 1.8	21.1 ± 1.5			
		D	33.6 ± 5.9	38.6 ± 12.0	34.5 ± 5.7	82.0 ± 44.0			
Naloxone 10 mg kg ⁻¹ (s.c.)	6	A	37.0 ± 2.2	47.9 ± 2.4*	46.3 ± 3.4*	46.0 ± 2.6*	47.9 ± 2.6*	46.0 ± 3.0*	47.0 ± 3.0*
		D	53.0 ± 4.9	68.0 ± 7.0	82.0 ± 6.0*	85.0 ± 7.0*	72.0 ± 6.0*	73.8 ± 3.5*	66.0 ± 4.8
Naloxone (5 mg) + acetorphan (10 mg)	11	A	30.8 ± 3.3	28.6 ± 7.0	29.7 ± 5.2	31.8 ± 4.0			
		D	45.6 ± 3.3	78.8 ± 19.0	72.9 ± 21.0	63.1 ± 10.7			
Thiorphan 0.15 mg kg ⁻¹ (i.v.)	6	A	15.8 ± 2.6	16.8 ± 1.5	21.0 ± 4.2	20.8 ± 2.6	19.8 ± 2.6	21.0 ± 4.8	20.8 ± 1.8
		D	56.0 ± 5.4	107.8 ± 48.0	113.6 ± 12.0*	94.0 ± 10.0*	108.0 ± 21.0*	105.0 ± 15.0*	85.0 ± 23.0
Thiorphan 1 mg kg ⁻¹ (i.v.)	8	A	37.0 ± 2.6	39.2 ± 6.4	38.0 ± 3.9	37.8 ± 3.4	42.0 ± 4.0	39.6 ± 5.0	37.0 ± 3.0
		D	54.2 ± 5.6	306.0 ± 88.0**	232.0 ± 46.0**	284.0 ± 62.0**	288.5 ± 78.0**	135.0 ± 48.0*	95.7 ± 27.0
Thiorphan 5 mg kg ⁻¹ (i.v.)	6	A	24.5 ± 2.0	31.1 ± 1.8*	31.4 ± 2.8*	32.8 ± 2.5*	28.8 ± 3.0	26.7 ± 1.7	26.0 ± 1.6
Naloxone (10 mg) + thiorphan (1 mg)	6	A	36.0 ± 4.0	45.8 ± 4.3	44.5 ± 6.0	40.6 ± 6.0	41.0 ± 4.5	40.3 ± 5.0	39.0 ± 5.2
		D	72.0 ± 11.0	96.6 ± 17.0	122.0 ± 37.0	116.0 ± 47.0	103.0 ± 17.0	99.0 ± 12.0	85.0 ± 6.0
Thiorphan 50 µg per rat (i.c.v.)	7	A	35.0 ± 4.0	32.6 ± 1.8	34.0 ± 1.4	43.8 ± 1.7*	45.3 ± 1.8*	47.7 ± 2.5*	47.0 ± 0.7*
		D	45.0 ± 3.5	46.4 ± 3.9	50.6 ± 8.1	52.4 ± 14.0	73.0 ± 10.0*	71.0 ± 9.0*	60.0 ± 5.4*

Each value of amplitude or duration represents the mean ± s.e.m of 6-13 rats per group. Naloxone was given 10 min before acetorphan or thiorphan.
*P < 0.05, **P < 0.01 compared with control value, Student's *t* test.

Table 2. Effects of thiorphan, [D-Ala²-Met⁵]-enkephalinamide (DAMEA) and naloxone administered alone or in combination on maximal amplitude (*A*; mm) or duration (*D*; s) on spontaneous uterine contractions in periparturient rats *in vitro*

Agent used	Number of experiments	Control	Treatment			
		- 15-0 min	1	2	4	
Thiorphan	6	<i>A</i> 26.6 ± 2.3	28.2 ± 1.0	35.5 ± 3.7*	39.2 ± 4.6*	
		<i>D</i> 49.0 ± 16.4	54.2 ± 9.0	52.0 ± 2.5	177.5 ± 2.5**	
DAMEA	7	<i>A</i> 27.0 ± 0.4	25.2 ± 3.3	28.1 ± 1.4	27.1 ± 3.5	
		<i>D</i> 72.8 ± 27.0	91.8 ± 12.0	125.9 ± 12.0*	232.8 ± 54.0**	
Naloxone	6	<i>A</i> 34.1 ± 2.5	35.5 ± 3.4	49.1 ± 4.1*	15.0 ± 3.5*	
		<i>D</i> 49.0 ± 5.9	50.0 ± 2.5	75.0 ± 3.5*	25.8 ± 2.1*	
Thiorphan (1, 2, 4) + DAMEA (1)	7	<i>A</i> 22.3 ± 2.7	25.0 ± 2.0	24.7 ± 2.9	30.9 ± 1.6	
		<i>D</i> 64.5 ± 16.0	162.6 ± 35.0	380.3 ± 94.1	848.0 ± 172.7	
Naloxone (2) + thiorphan (4)	6	<i>A</i> 29.3 ± 4.4		39.0 ± 10.0		
		<i>D</i> 48.9 ± 16.0		72.4 ± 18.0		
Naloxone (2) + thiorphan (4) + DAMEA (1)	6	<i>A</i> 22.3 ± 2.7			17.0 ± 2.6	
		<i>D</i> 64.6 ± 16.0			27.3 ± 6.0	

Each value of amplitude and duration represents the mean ± SEM of 6-7 animals per group. Each drug was given in increasing concentrations and each concentration was successively multiplied by a factor of 2. Thiorphan (10, 20 and 40 µmol l⁻¹), DAMEA (3.7, 7.4 and 14.8 mol l⁻¹) and naloxone (13, 26 and 52 mol l⁻¹).

P* < 0.05, *P* < 0.01 compared with the control value (Student's *t* test).

declined 1 day before parturition (Ottlecz *et al.*, 1991), suggesting that during pregnancy, it may contribute to the prevention of parturition by regulating the activity of uterine motility by inactivating enkephalin and other circulatory regulatory peptides that have an important role in uterine motility, such as oxytocin (Adjroud, 1988; Ottlecz *et al.*, 1991), which is also a substrate for enkephalinase (Johnson *et al.*, 1984). The pregnant rat uterus also contains large amounts of mRNA encoding proenkephalin (Jin *et al.*, 1988), which appears to increase in the mouse uterus during pregnancy (Rosen *et al.*, 1990), but the mechanism by which enkephalins act on uterine muscle is not known. Enkephalin stimulates the amplitude of contractions in cultured chick embryo heart muscle cells by stimulating adenylate cyclase and increasing cAMP concentrations (Laurent *et al.*, 1986), and exerts a positive inotropic effect by augmenting Ca²⁺ influx (Laurent *et al.*, 1986). Enkephalin has been shown to reduce adenylate cyclase activity in rat vas deferens (Bhoola and Pay, 1986). Naloxone acts as an antagonist of enkephalin in the brain (Otero *et al.*, 1993; Coffield and Miletic, 1993) and the periphery (Maas and Leek, 1985). In the present study, naloxone reduced the effects of enkephalinase inhibitors and the met-enkephalin analogue on the motility of the periparturient uterus *in vivo* and *in vitro*, suggesting that opioid peptides act locally in the uterus via opioid receptor systems, which have been identified in the pregnant rat uterus (Baraldi *et al.*, 1985). This antagonistic effect of naloxone is in agreement with other results obtained on the motility of isolated rat and mouse vas deferens (Aoki *et al.*, 1986; Lingyuan *et al.*, 1986), isolated dog intestine (Burks *et al.*, 1982) and *in vivo* sheep reticulo-rumen (Maas and Leek, 1985). However, naloxone (10 mg kg⁻¹) alone markedly increased the duration and amplitude of uterine contractions in rats. Similar excitation by naloxone is observed in reticulo-ruminal motility in con-

scious sheep (Maas and Leek, 1985). The mechanism by which naloxone stimulates uterine motility is not clear. Its excitatory effect could be explained by the fact that it is an opioid antagonist that crosses the blood-brain barrier easily (Calligan and Burks, 1983) and acts on other components involved in the excitation of periparturient rat uterus such as oxytocin. This hypothesis is supported by the finding of Hartman *et al.* (1984) that plasma concentrations of oxytocin, measured during parturition, are raised in rats treated chronically with naloxone, and the observation that the uterotonic effect of oxytocin is potentiated in the presence of naloxone (Adjroud, 1988). In contrast to experiments *in vivo*, naloxone showed a biphasic effect on preparations *in vitro*. At a lower dose it exhibited a stimulatory effect and at a higher dose it was inhibitory. Similar paradoxical effects of naloxone are reported from analgesic tests in mice (Noble *et al.*, 1994).

The results of present study suggest that the uterotonic effects of enkephalinase inhibitors and met-enkephalin in the periparturient uterus are due to the presence of specific local enkephalin receptors that modulate myometrial activity. These local enkephalinergic receptors can be blocked by naloxone and by part of a local enkephalinergic system, which remains to be defined.

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