Action of naloxone and acetorphan, on uterine contractions induced by oxytocin in periparturient rat in vivo

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SUMMARY

The effects in vivo of acetorphan, an enkephalinase inhibitor and naloxone, an opiate antagonist on the oxytocin- induced myometrial mechanical activity were studied at day 21 of pregnancy in rats. Acetorphan, 10 mg kg $^{-1}$ (i.v.) increased (344%) spontaneous uterine contractions duration only at 0-10 min period, but did not modify amplitude. This increase in the duration was blocked by naloxone 5 mg kg $^{-1}$ (s.c.), which had no effect on the amplitude or duration of contractions when given alone. Oxytocin (OT), 1, 10 and 100 mIU (i.v.) stimulated in a dose dependent manner, spontaneous uterine contractions duration but did not alter the amplitude of contractions. Blocking the opioid peptides by naloxone, 5 mg kg $^{-1}$ (s.c.) injected 10 min before OT strongly potentiated the effect of the lowest dose of OT (1 mIU) by about of 598% and doubled the amplitude of contractions during the 30 min observation period. When acetorphan, 10 mg kg $^{-1}$ (i.v.) administered 30 min before the lowest dose of OT studied, 1 mIU (i.v.), It induced a slight increase by about 88% in uterine contractions duration produced by OT alone. The amplitude remained unchanged. These results suggested that uterine contractions induced by OT in late pregnancy in rats might be regulated by enkephalinergic and enkephalinasic systems.

RESUME

Les effets de l'acetorphan, un inhibiteur de l'enképhalinase et de la naloxone, un antagoniste des opiacés sur l'activité mécanique myométriale induite par l'ocytocine, sont étudiés in vivo chez la rate préparturiente au 21 ^{e} jour de gestation. L'acetorphan (10 mg kg $^{-1}$, i.v.) augmente de 344% la durée des contractions utérines spontaneés pendant seulement les 10 premières minutes, mais ne modifie pas l'amplitude. Cette augmentation de la durée est bloquée par la naloxone (5 mg kg $^{-1}$, s.c.), laquelle, lorsqu'elle est administrée seule, n'a aucun effet ni sur l'amplitude ni sur la durée des contractions. L'ocytocine (1, 10 et 100 mUI, i.v.) stimule la durée des contractions utérines spontanées de façon dose-dépendante mais n'altère pas l'amplitude. Le blocage des peptides opiacés endogènes par la naloxone (5 mg kg $^{-1}$, s.c.) potentialise de 598% l'effet de la plus faible dose (1 mUI) d'ocytocine sur la durée des contractions et double celui de l'amplitude durant les 30 minutes de la période d'observation. lorsque l'acetorphan (10 mg kg $^{-1}$, i.v.) est administré 30 min avant la plus faible dose d'ocytocine (1mUI, i.v.), il induit un accroissement non significatif de 88% de la durée des contractions produites par l'ocytocine seule. L'amplitude demeure inchangée. Ces résultats suggèrent que les contractions utérines induites par l'ocytocine chez la rate préparturiente pourraient être régulées par des systèmes enképhalinergique et enképhalinasique.

INTRODUCTION

OT stimulates contractions of the uterus at parturition. Similarly met- enkephalin increased spontaneous uterine contractions in late pregnancy (Adjroud, 1995). On the other hand, met-enkephalin concentrations (Martin & Voigt, 1981) and opioid receptors (Falke & Martin, 1986) coexist with OT in nerve terminals of rat neurohypophysis. Moreover, the enkephalinase which hydrolyses the met and Leu-enkephalin is also presumes to cleave human placenta OT [Johnson et al., 1984), such enzyme is also presents in the pregnant rat and human uterus [Otllecz et., 1991; Germain et al., 1994). The actions of endogenous opioid peptides on the release of OT from neurohypophesis are very contradictory. Inhibitory effects of opioid on the release of OT (Pamford et al., 1993) or failure to detect an effect (Nordmann et al., 1986) were reported. Therefore the principal objective of this study is to attempt to provide an eventual interaction between enkephalin and OT on spontaneous uterine contractions in the rat at day 21 of pregnancy (term at day 22) using naloxone, an enkephalin antagonist, and acetorphan, a lipophilic potent enkephalinasic inhibitor.

Materials and Methods Animals

Adult Female Wistar rats (250-350 g body mass) were kept in a lighting schedule of 12 h light : 12 h darkness at 23 ± 1°C with free access to food and water. Animals were used at day 21 of pregnancy. The average length of gestation in breeding colony in our department was 22 days.

Measurement of uterine motility in vivo

Each animal was anaesthetized with sodium pentobarbital (45 mg Kg^{-1}, i.p.; Abbott Laboratories, St Remy sur Avre) and the trachea was cannulated to assist respiration. The abdomen was opened using midline incision and the medial part of one uterine horn was linked to an isometric strain gauge connected to an ink polygraph. The uterus was securited to the strain gauge by a cotton thread passed between the myometrium and the blood vessels of the mesometrial membranes. The cervical end of the part of the uterus that was used for recording was securited to a vertical fixed metal rod to maintain isometric conditions. A standard tension of 1 h 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 appropriate route and 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.

Treatment regimens.

Sources and preparation of solutions of acetorphan and naloxone were as stated elsewhere (Adjroud, 1995). OT (Syntocinon 5 IU, Sandoz) was purchased from Sigma Chemical Company (St Louis MO) and was dissolved in sterile saline. OT was given i.v. at 1, 10 and 100 mIU and acetorphan was given i.v. at 10 mg kg^{-1} body mass in 0.3 ml. Treatment with naloxone was used to attempt to block the effects of the enkephalinase inhibitor and to study its influence upon the uterotonic of OT-induced. Naloxone was given s.c. at 5 mg Kg^{-1} body mass in 0.3 ml 10 min before i.v. acetorphan (10 mg kg^{-1} body mass) or OT (1 mIU).

Measurement of the spontaneous uterine contractions .

Variations in myometrial mechanical activity were evaluated in terms of the duration (sec) and amplitude (mm) of contractions as described earlier (Adjroud, 1995). Briefly the amplitude and duration of each contraction during the control period were calculated. Group of 6-13 animals were assigned to each treatment. Each recording of the treatment lasted 30-60 min. This time was divided into 10 min periods. The means ± SEM of the amplitude and duration of contraction of each group of treated animals were calculated at 10 min periods during 30-60 min of recording

statistical analyses

Data for each group of experiments (n= 6-13) were analysed by analysis of variance and expressed as mean ±SEM and represented in Table 1 and Fig. 1. Significant differences between the treated group mean and its own control were performed by Student's "t" test. Differences were considered to be significant if P < 0.05.

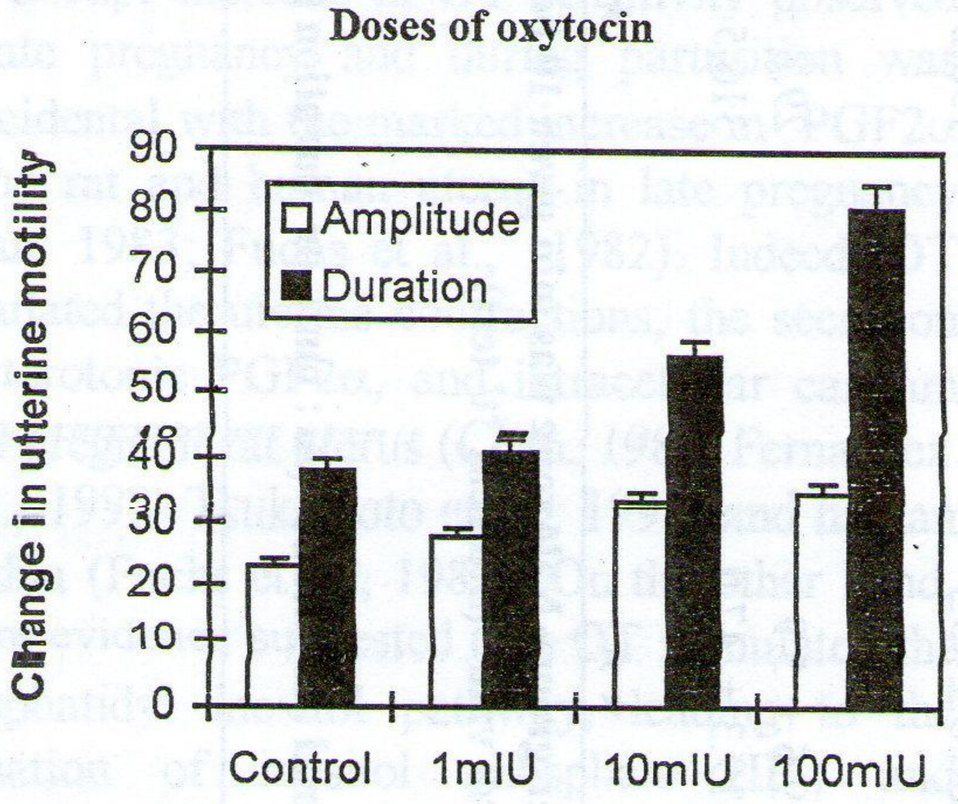

Fig.1 Effects of graded doses of oxytocin on uterine motility in periparturient rat *in vivo*. Each value of amplitude and duration represents the mean ± SEM of 8 rats. ★ P<0.05 compared with control value (Student 't' test)

Results

Effects of OT.

OT administered at graded doses (1. 10 and 100 mIU, i.v.; n = 8) produced during each 30 min for each observation an increase mainly in the duration of uterine contractions in a dose dependentntnt-manner, 8.48 ± 11.73%, 48.79 ± 12.6% and 113 ± 10% (Fig. 1). The maximum response (43%, P<0.05) is achieved with the higher dose studied, 100 mIU compared to 10 mIU. The maximum amplitude progressively but no significantly increased after treatment with each dose (21.2 ± 11%, 43 ± 13.73% and 50 ± 9.4% Fig.1) respectively, compared to control value

Effect of tne combination of OT and naloxone.

Naloxone 5 mg Kg^{-1} ,s.c, (n=6). given 10 min before the lowest dose of OT studied 1 mUI (i.v.) tonically increased the duration of uterine contractions by about 598% (P <0.001) during 30 min observation period, compared to the control value. This association also significantly increased by about 89% the amplitude of uterine contractions over 30 min. Moreover, naloxone 5 mg kg^{-1}, s.c., (n= 8) when given alone did not induce any significant change (P>0.05) in the amplitude or duration of uterine contractions over 30 min .

Effects of acetorphan alone or in combination with naloxone

Acetorphan 10 mg kg^{-1}, i.v., (n=13) had no effect on amplitude but induced a significant increase (344%, P<0.01) in the duration of contractions during the first 10 min after administration. The duration of contractions had returned to pretreatment levels by the 20-30 mins ampling period. This effect was not seen when naloxone 5 mg Kg^{-1} (n=11) was given 10 min before acetorphan.

Effects of the combination of acetorphan and OT.

Acetorphan, 10 mg kg^{-1}, i.v, (n= 8) administered 30 min before the lowest dose of OT studied 1 mU (i.v.) modestly but not significantly augmented the increase about 88% in the duration of contractions during the 30 min observation period compared to OT alone. The amplitude did not change.

Discussion

In both humans and rats, myometrial sensitivity to OT increased through late gestation in parallel with an increase in myometrial OT receptor concentrations (Fuchs et al., 1982; 1983; Chan & Chen 1992). The local concentration of OT , mRNA of OT receptors and OT receptors in the uterus of the late gestation of pregnant rat were regulated by sex steroids, and correlated closely with the ratio of serum estrogen to progesterone concentration. Estrogen increased OT binding, mRNA of OT receptors and OT concentrations in normal and ovariectomized pregnant rats, and progesterone inhibits the estrogen-induced rise (Fuchs et al., 1983; Larcher et al., 1995; Fan et al., 1996). The present data demonstrated that OT, one of the most potent uterotonic agents in the last stages of pregnancy (Fuchs et al., 1983), administered i.v. to rats at day 21 of pregnancy

Agents used, dose		Control	After treatment		
		-30-0 min	0-10 min	10-20 min	20-30 min
Acetorphan	13	A = 30.0 ± 3.0	31.5 ± 2.8	30.2 ± 1.5	28.6 ± 3.5
10 mg kg $^{-1}$ (i..v.)		D = 61.9 ± 14.0	271.0 ± 74 ★	94.1 ± 24.0	68.8 ± 15.0
Naloxone	8	A = 22.0 ± 3.8	22.7 ± 4.8	24.0 ± 1.8	21.1 ± 1.50
5 mg kg $^{-1}$ (s.c.)		D = 33.6 ± 5.9	38.6 ± 12.0	34.5 ± 5.7	82.0 ± 44.0
Oxytocin	8	A = 23.2 ± 1.9	22.2 ± 3.4	21.2 ± 3.0	22.5 ± 3.0
1 mUI (i.v.)		D = 76.2 ± 11.5	73.2 ± 6.7	91.7 ± 16.3	72.6 ± 8.5
Naloxone (5 mg)	11	A = 30.8 ± 3.3	28.6 ± 7.0	29.7 ± 5.2	31.8 ± 4.0
+ acetorphan (10 mg)		D = 45.6 ± 3.3	78.7 ± 19.0	72.9 ± 21.0	63.1 ± 10.7
Acetorphan (10 mg)	8	A = 23.2 ± 1.9	22.9 ± 2.3	23.6 ± 2.1	22.8 ± 1.7
+ oxytocin (1 mUI)		D = 76.2 ± 11.5	163.9 ± 66.9	138.6 ± 43.7	138.2 ± 37.9
Naloxone (5 mg)					
+ oxytocin (1 m UI)	6	A = 15.7 ± 1.1	29.3 ± 4.0	29.6 ± 5.9	29.8 ± 4.2
		D = 55.3 ± 9.2	421.8 ± 45.0	419.3 ± 47.0	316.5 ± 59.6
			★★	★★	★★

O. Adjroud. Each value of amplitude or duration represents the mean ± SEM of 6-13 rats per group. Naloxone was min given 10 before acetorphan or oxytocin, and acetorphan was given 30 min before oxytocin. * P<0.01 P< 0.001 compared with control value (Student 't' test)**

Table 1
Effects of naloxone, acetorphan and oxytocin administered alone or in association on maximal amplitude (A : mm) or duration (D : sec) on spontaneous uterine contractions in periparturient rats *in vivo*.

(one day before parturition) induced an increase in uterine motility duration in a dose dependent manner. This result is in accordance with those reported by Tsukamoto et al (1991). OT might stimulate myometrial contractions via several mechanisms. It has been shown that the stimulation of uterine muscle in late pregnancy and during labour seems mediate by prostaglandins (PG) and intracellular calcium. The abrupt increase in OT sensitivity observed in late pregnancy and during parturition was coincidental with the marked increase in PGF2α in the rat and human uterus in late pregnancy (Chan, 1983; Fuchs et al., 1982). Indeed, OT stimulated the uterine contractions, the secretion of uterotonic PGF2α, and intracellular calcium from pregnant rat uterus (Chan, 1983, Fernandez et al., 1992; Tsukamoto et al., 1991) and human decidua (Fuchs et al., 1982). On the other hand, recent evidence suggested that OT stimulated the phosphatidyl inositol pathway, leading to the formation of inositol phosphate (IP_3) and diacylglycerol , second messengers. IP_3 stimulated uterine motility, increased release of intracellular calcium (Savineau et al., 1990) and lead to the synthesis of PGF2α within myometrial cells (Schrey et al., 1988). The PG released in the human decidua may diffuse into adjacent myometrium and potentiated OT-induced contractions (Fuchs et al., 1989). Moreover, acetorphan; an enkephalinase inhibitor induced a significant increase in duration of uterine contractions in pregnant rat, this increase was not appeared in presence of naloxone. This observation suggested the involvement of the enkephalinasic and enkephalinergic systems in the modulation of uterine motility in late pregnancy. These findings were previously confirmed (Adjroud, 1995) using a preparation of isolated uterine strip, where met-enkephalinamide or thiorphan, an other enkephalinase inhibitor administered alone or in combination significantly increased the uterine contractions duration, and this increase was blocked by naloxone. Furthermore, blocking the action of opiod peptides with naloxone further potentiated the effect of the submaximal dose of OT on spontaneous uterine contractions. This study demonstrated that naloxone exerts positive effects on the responsiveness of pregnant rat uterus to OT, suggesting inhibitory actions of endogenous opioid peptides. This hypothesis is agreed with the observations demonstrated that morphine inhibited the firing of magnocellular OT neurones in the female rat, inhibiting OT secretion and then plasma OT concentrations (Pamford et al., 1991). Whereas naloxone stimulated the firing rate of putative OT neurones in the female rat (Pamford et al., 1991) and increased the plasma OT concentrations in the pregnant rat (Douglas et al., 1993).This is however completely contradictory to the effect of acetorphan reported here. Indeed, acetorphan had no a significant effect on uterine contractions duration induced by a submaximal dose of OT tested, although there was a tendency towards an increase. This results which failed to demonstrate in vivo an inhibitory effect of enkephalin on OT agree however with those of Nordmann et al (1986) who failed to observe an inhibitory effect of enkephalin on OT release in vitro, and the slight increase in uterine duration induced by the association of OT with acetorphan was in part in accordance with the observation reported by Ottlecz et al., (1991) where phosphoramidon, an enkephalinase inhibitor, produced a marked potentiation of OT-induced- uterine contractions in pregnant rats in vitro.

The results obtained with association of acetorphan and OT prompted us to develop a preparation of isolated uterine strip. This has the advantage of studying the mechanism in absence of other tissues such as hypophysis and placenta.

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