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The Effect of Oxytocin Antagonist on Uterus in Response to Exogenous Oxytocin

This study was performed to determine the action mode of oxytocin antagonist. In Study 1, the duration of in vivo action of oxytocin antagonist I (AI) was examined. After infusing AI, oxytocin was given and repeated every hour for 5 hr. Uterine activities were monitored with a polygraph. Study 2 determined the effect of AI on uterine oxytocin receptor number (Rn) and binding affinity (Kd). AI treated rats were sacrificed at 0.5 and 4 hr later for receptor assay. In Study 1, the uterine contractile response to oxytocin was significantly inhibited ($p<0.05$) compared to controls at five min, 1 and 2 hr after injection of AI. No differences in response were detected compared to controls ($p>0.05$) at later hours. In Study 2, no differences ($p>0.05$) between the AI and control animals in either oxytocin receptor number or binding affinity was found. These data suggest that the major mode of AI action is via competitive inhibition at the uterine oxytocin receptor and not by altering receptor number or binding affinity. AI is suggested to have the potential of being a potent and specific tocolytic agent for prevention of preterm labor in human.

Key Words: *Oxytocin, Oxytocin Antagonist; Uterus; Receptor; Labor*

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INTRODUCTION

The role of oxytocin (OT) during parturition in primates has not been throughly defined due to inconsistent reports on the plasma oxytocin levels. Some studies support an involvement of oxytocin during the labor process as demonstrated by an increase in the myometrial oxytocin receptor number (1), increase in sensitivity of uterine myometrium to oxytocin (2) and increase in uterine oxytocin gene expression (3). Furthermore, preterm labor is associated with a significant increase in myometrial sensitivity to oxytocin while post-term labor is associated with decreased sensitivity (4). If oxytocin is important in preterm labor, an oxytocin antagonist would be of great clinical significance.

In fact, preterm labor is the major cause of fetal mortality and morbidity even in developed countries (5). However, no effective medicine is currently available to treat these problems. Commonly used tocolytic agents include beta-agonists, magnesium sulfate, prostaglandin inhibitors and calcium blockers. However, they are not very effective and have significant side effects. An ideal tocolytic should be efficacious, specific and have no side effects. An oxytocin antagonist is potentially such a tocolytic. Initial clinical trials with Atosiban, an oxytocin antagonist, are encouraging (6-9).

There were reports that AI inhibits in vitro and in vivo uterine contractions in response to exogenous oxytocin in the rat (10, 11), baboon (12, 13) and human (10, 11). It is assumed that AI blocks uterine contractions via competitive inhibition at the oxytocin receptor. But, it is not clear whether AI has other unknown effects on the receptor. So far, there have been no studies that evaluated the action duration of AI in vivo. Therefore, the present study was designed to examine the action mode of AI after a single bolus injection in vivo and determine whether in vivo infusion of AI alters uterine oxytocin receptor number and/or binding affinity in the estrous rat.

MATERIALS AND METHODS

Animal subjects

Holtzman rats weighing between 200 to 240 g were

used in this study. Animals were housed in rooms with controlled light cycles (14 hr light and 10 hr dark, lights on 6 a.m.) and given food and water as desired.

Oxytocin antagonist

The synthesis, bioassay potency and initial efficacy screening test for AI have been previously reported (10, 12). The chemical structure of AI is [Beta-mercapto-beta, beta-cyclopentamethylene propionic acid[1], D-Trp[2], Phe[3], Ile[4], Arg[8]]-oxytocin.

Animal cannulation

Rats in natural estrus were anesthetized with chloral hydrate (500 mg/kg) intraperitoneally. A catheter (PE-50) was placed into the jugular vein for infusing AI (5 μg) or saline (control) as a bolus injection. To monitor uterine contractions, a PE-50 balloon-tipped and water-filled cannula was placed into one uterine horn at the ovarian end. Integrated intrauterine pressure changes were measured over 10 min using Grass Polygraph and recorded with a Gould P23id pressure transducer (both from Grass Instruments, Quincy, MA). Animals were sacrificed by overdosing with chloral hydrate.

In vivo oxytocin challenge test in Study 1

The experimental treatments were saline and 5 μg of AI. Five min after infusing AI, 100 mU of oxytocin was given and repeated every hour up to 5 hr. The 5 min time point is referred to as the 0 hr in Fig. 1. Six animals were used at each time point.

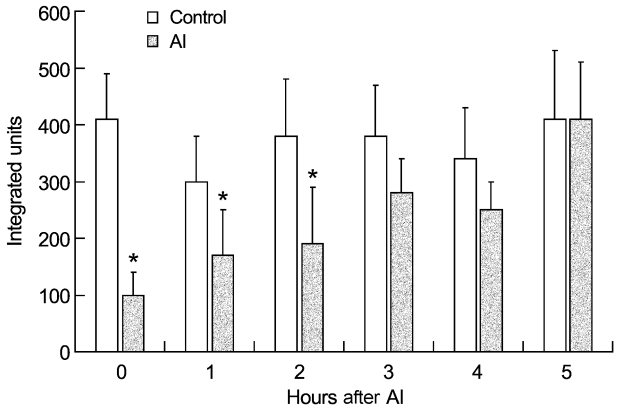

Fig. 1. Illustrated is the in vivo uterine response to 100 mU of oxytocin given every hour for 5 hr in nonpregnant estrous rat following infusion of 5 μg of the oxytocin antagonist, AI or saline. *significantly different from control ($p<0.05$). N=6 at each time point

Oxytocin receptor assay in Study 2

Uterine tissue was obtained at 0.5 and 4 hr following 5 μg AI or saline intravenous bolus administration. These time points were chosen based on the results of in vivo oxytocin challenge test shown in Fig. 1. At 0.5 hr, AI significantly inhibited the uterine response to exogenous OT, but efficacy gradually disappeared by 4 hr. Six animals were used for each experimental group.

The methodology of the assay has been described previously (11). In brief, the uterine tissue from sacrificed estrous rat was cut into small pieces (3 mm×3 mm) with scissors, frozen on dry ice, and stored at -70℃ until analysis. The uterine tissue was homogenized in 5 mM Tris buffer containing phenylmethylsulfonyl fluoride (PMSF) over ice. The homogenate was filtered through a gauze and filtrate was centrifuged at 1,000 *g* for 15 min at 4℃. The supernatant was centrifuged at 40,000 *g* for 30 min and the cell membrane containing pellet resuspended in 10% sucrose. To perform density gradient centrifugation, the 10% sucrose membranes were placed on top of 35% sucrose and centrifuged for 30 min in a swing bucket rotor at 105,000 *g*. The membranes at the interface of the 10/35% sucrose were removed and resuspended in Tris buffer containing EDTA for 30 min. This procedure removes divalent cations and results in dissociation of endogenously bound oxytocin to the receptor. The mixture was centrifuged for 15 min at 100,000 *g* and the pellet was resuspended in Tris, PMSF, Mg buffer by sonication.

The binding assay consisted of 0.05 mL (10,000 cpm) of d(CH^2)$_5$[Tyr(Me)^2, Thr^4, Orn^8, [^{125}I]Tyr^9-NH^2]-Vasotocin (DuPont Company, Wilmington, DE; 2200 Ci/mmol), 0.05 mL of oxytocin (Calbiochem Behring Corp., LaJolla, CA), membrane oxytocin receptor and 50 mM Tris buffer containing 0.5% trypsin inhibitor, 100 mM PMSF, 1% bovine serum albumin and 20 mM MgCl. Incubation in ultraclear minitubes (5×41 mm) was performed for 30 min at 30℃. This was followed by centrifugation for 30 min at 105,000 *g*. The resulting membrane pellet was counted for cpm in a gamma counter.

The receptor number and dissociation constants were determined by nonlinear curve-fitting techniques using McPherson's EBDA (14) and Munson and Rodbard's LIGAND (15) programs for saturation and competition analysis.

Data analysis

Data from Study 1 were analyzed by repeated measures of analysis of variance, and when significant, differences were determined by Duncan's Multiple Range test (16). Data from Study 2 were analyzed by two way analysis of variance with interaction (16).

Table 1. The effect of oxytocin antagonist on receptor number and dissociation constant

	0.5 hr		4 hr		Statistical significance
	Saline control (n=6)	Oxytocin antagonist I (n=6)	Saline control (n=6)	Oxytocin antagonist I (n=6)	
Rn (fmole/mg)	3,800±5.5	4,250±3.2	3,275±1.5	4,132±0.8	Not significant
Kd (nM)	0.8±2.5	0.77±3.4	1.37±2.9	1.24±1.9	Not significant

All values are mean±SEM.
Rn, oxytocin receptor number; Kd, dissociation constant

RESULTS

The inhibition of oxytocin-induced uterine contractions following 5 μg AI bolus infusion in the estrous rat on in vivo uterine contractions at 0, 1, 2, 3, 4 and 5 hr is shown in Fig. 1. AI significantly inhibited the uterine response to oxytocin at 0, 1 and 2 hr ($p<0.05$). However, from 3 to 5 hr the response to oxytocin was no longer different from controls ($p>0.05$). Based on this result, we designed Study 2 to look at the oxytocin receptor number and dissociation constant at the 0.5 and 4 hr. Differences in the main effects (treatment and time) or interaction were not significant ($p>0.05$). The results of treatment (AI vs control) on oxytocin receptor number and binding affinity are presented in Table 1.

DISCUSSION

The results of this study showed that 5 μg bolus infusion of AI, oxytocin antagonist, inhibits the uterine contractile response to 100 mU of exogenous oxytocin for up to 2 hr in the estrous rat. It also showed no significant effect on oxytocin receptor number and oxytocin receptor binding affinity when compared with controls. These data suggest that in vivo tocolytic activity of AI is fairly long acting, probably via competitive inhibition of the oxytocin receptors. It could be possible that receptors were modulated by AI. However, the in vitro oxytocin bioassay studies do not support this possibility since the rat uterus returns to normal responsitivity after washing out the AI from the organ bath with new buffer (11). The in vitro activity of AI may depend on events other than receptor binding such as a long half life.

The development of a potent oxytocin antagonist would be useful for studying the contribution of endogenous oxytocin to nocturnal and labor uterine contractions during pregnancy. The maximum oxytocin receptor number occurs at the end of pregnancy in the rat (1), correlating with the increase in uterine sensitivity to oxytocin at the time of labor. Although Lefebvre et al. (3) demonstrated that oxytocin mRNA in the uterus and placenta increased as delivery approaches, the expression of oxytocin appears to be minimal thus questioning its contribution to uterine activity at delivery.

AI is about 2.5 times more potent than Atosiban as determined by in vitro rat oxytocin bioassay and oxytocin receptor assay (11). Its potency was further illustrated when five different oxytocin antagonists were compared (13). AI is a potent inhibitor of oxytocin-induced uterine contractions as evidenced by inhibition of uterine contractions induced by exogenous oxytocin in vitro and in vivo, by inhibition of milk let-down and by disruption of labor (10, 12). Furthermore, AI inhibited in vitro contractions induced by oxytocin in myometrial tissue obtained by C-section at term pregnancy in human (10).

Based on the current results with a report that states AI is a potent inhibitor of nocturnal and labor uterine contractions in the pregnant baboon (12), it is suggested that AI has the potential of inhibiting preterm labor in human.

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