韓國家畜繁殖學會誌 18(2) : 101~104(1994)
Korean J. Animal Reprod.

In Vivo Effect of Oxytocin Antagonist Ⅰ on an Oxytocin Challenge Test in the Rat

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흰쥐를 이용한 옥시토신 자극검사에 대한 옥시토신 길항제-Ⅰ의 생체투여 효과

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요 약

임신기간중 oxytocin의 역할을 규명하기 위해 그리고 조기분만 진통의 억제제로서 수많은 강력한 oxytocin antagonist들이 개발되고 있다. 본 연구는 발정된 흰쥐를 사용하여 oxytocin antagonist Ⅰ (AI)이 control과 비교하여 자궁에 어떠한 활동을 보이는지를 알아보는 것이 주 목적이었다. AI과 control로서 saline을 주입하기 위해 경정맥에 cannula를 수술하여 집어 넣었고, 또 다른 cannula는 자궁활동을 측정하기 위해 자궁각에 집어 넣었다. 자궁수축상황은 Grass Polygraph를 사용하여 측정하였고 수축활동은 10분동안의 integrated area를 계산하여 측정되었다. 5㎍의 AI을 주입한 5분후 100mU의 oxytocin이 주입되었고 이 oxytocin주입은 매시간 5시간 동안 계속되었다. AI이 주입된 5분후, oxytocin에 대한 자궁의 수축반응은 control에 비해 77% 감소되었다($P<0.05$). AI 주입 2시간 후에는 그 감소가 control에 비해 54%였다. 그러나 3시간 이후부터 AI은 control과 어떤 유의한 차이를 보이지 않았다. AI이 인간의 조기분만진통을 방지하는데 사용될 수 있다는 잠재 가능성을 본 연구에서 확인하였다.

Ⅰ. INTRODUCTION

Preterm birth is the major cause of fetal mortality and morbidity in developed countries (Higby et al., 1993). However, no drug is currently available to effectively treat this problem. 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 antagonists is potentially such a tocolytic. Initial clinical trials with the oxytocin antagonist, Atosiban, are encouraging(Hahn et al., 1987; Akerlund et al., 1987; Andersen et al, 1989; Goodwin et al., 1994). Potent oxytocin antagonists have been developed by our laboratory including one referred to as oxytocin antagonist I(AI) (Wilson et al., 1990a; Wilson et al., 1990b). Our laboratory has reported that AI

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inhibits *in vitro* and *in vivo* uterine contractions in response to exogenous oxytocin in the rat and baboon (Wilson el al., 1990a; Wilson et al., 1990b). However, none of these studies evaluated the duration of action of AI *in vivo*.

Preliminary studies suggested AI might have a prolonged duration of action than anticipated from *in vitro* studies. Therefore, the present study was designed to examine the duration of action of AI after a single bolus infection *in vivo* in the estrous rat.

Ⅱ. MATERIALS AND METHODS

1. Animals

Rats(Holtzman Co, Madison, Wsiconsin)weighing between 200 to 240 gms were used in this study. The rats were housed in rooms with controlled light cycles(14 hours light and 10 hours dark, lights on 6 a.m.) and given food and water as desired. This protocol was approved by the Animal Care Committee at the University of Illinois.

2. Oxytocin antagonist

The synthesis, bioassay potency and initial efficacy screening tests for AI have been previously reported(Wilson et al., 1990a; Wilson et al., 1990b). The chemical structure of AI is [Beta-mercapto-beta, beta-cyclopentamethylene propionic acid[1], D-Trp[2], Phe[3], Ile[4], Arg[8]]-oxytocin.

3. *In vivo* oxytocic bioassays

Response of the estrous rat uterus *in vivo* to oxytocin and oxytocin antagonist was tested as described Wilson et al. (1990a). Rats were anesthetized with chloral hydrate(500mg/kg) intraperitoneally and a balloon-tipped, water filled polyethylene catheter(PE-SO) was implanted through a small incision at the ovarian end of the uterus and tied in place for assessing uterine activity. uterine contractions were monitored with polygraph (Grass Instruments)and recorded with a Gould P23 ID pressure transducer. The uterine contractile activity was determined as the integrated area under curve for 10 minutes. A PE-50 polyethylene cannula was placed in the jugular vein for infusing oxytocin and AI.

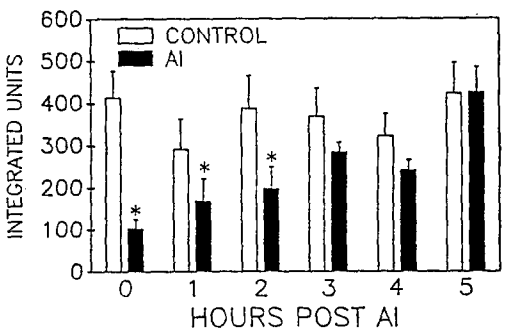

Fig. 1. Illustrated is the *in vivo* uterine response to 100mU of oxytocin given every hour for 5 hours in nonpregnant estrous rats following infusion of 5 μg of the oxytocin antagonist, AI, or control.

* Different from control ($p<0.05$)

The experimental treatements were control and 5 μg of AI. Five minutes after infusing AI 100 mU of oxytocin was given and repeated every hour up to 5 hours. The 5 minute time point is referred to as the 0 hour in Fig. 1. Data were analyzed by repeat measures analysis of variance, and when significant, defferences were determined by Duncan's Multiple Range test (Norusis, 1988).

Ⅲ. 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 hours is shown in Fig. 1. AI significantly inhibited the uterine response to oxytocin at 0, 1 and 2 hours($p<0.05$). However, from 3 to 5 hours the response to oxytocin was no longer different from controls ($p>0.05$)

Ⅳ. DISCUSSION

The results of this study show that 5 μg bolus injection of the oxytocin antagonist, AI, inhibits the uterine contractile response to 100mU of exogenous oxytocin for up to 2 hours in the estrous rat. These data suggest that *in vivo* tocolytic activity of AI is fairly long acting. This is somewhat unexpected since in the *in vivo* oxytocin bioassay AI's inhibitory effect is rapidly reversed after washing out the organ bath with new buffer. The results suggest that AI's activity *in vitro* may depend on events other than receptor binding such as a long-half life.

AI is about 2.5 times more potent than Atosiban as determined by *in vitro* rat oxytocic bioassay and oxytocin receptor assay(Pak et al., 1994). Atosiban is and oxytocin antagonist currently undergoing clinical trials for inhibition of preterm labor throughout the world. The results of these studies are encouraging. Based on the results of the present study, and reports form our laboratory that AI is a potent inhibitor of nocturnal and labor uterine contractions in the pregnant baboon(Wilson et al., 1990b), these data support the suggestion that AI has the potential of inhibiting preterm labor in humans.

Ⅴ. ABSTRACT

The purpose of the present study was to examine the *in vivo* activity of oxytocin antagonist I (AI)in the nonpregnant estrous rat. Cannulas were placed in the jugular vein for infusing compounds and a water-filled balloon-tipped cannula placed in one uterine horn for assessing uterine activity. Uterine contractions were monitored with a Grass Polygraph and contractile activity determined as the integrated area for 10 minutes. Five minutes after infusing 5 μg of AI, 100mU of oxytocin was given as an in bolus injection and repeated every hour for 5 hours. At five minutes, 1 and 2 hours after injection AI the uterine contractile response to 100 mU of oxytocin was significantly inhibited compared to controls($p<0.05$). At 3, 4 and 5 hours no differences in response were detected compared to controls($p>0.05$). These results in conjunction with other reports from our laboratory suggest that AI has the potential of being a potent and specific tocolytic for prevention of preterm labor in humans.

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