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Tocolytic Effect of Morphine via Increased Metabolic Clearance of Oxytocin in the Baboon

Young Hoon Bai[1], Sok Cheon Pak[2], Bum Chae Choi[3], and Laird Wilson Jr.[4]

[1]Research Division of Biological Science, College of Medicine, Chosun University, Gwangju, Korea;
[2]Hamilton College of Oriental Medicine, Hamilton, New Zealand;
[3]Creation and Love Women's Hospital, Gwangju, Korea;
[4]Department of Obstetrics and Gynecology, University of Illinois at Chicago, Chicago, IL, USA.

Morphine is known to inhibit nocturnal uterine contractions in several animal models, and oxytocin is known to be a primary causative factor of uterine contractions. The purpose of the present study was to determine the tocolytic effect of morphine in relation to the pharmacokinetics of oxytocin, after a bolus injection of oxytocin. The metabolism of oxytocin was investigated during the third trimester in baboons. Four animals were placed on a tether system with venous and arterial access, including continuous uterine monitoring. Plasma oxytocin levels were determined by radioimmunoassay after extraction with petroleum ether/acetone. Morphine consistently increased the metabolic clearance rate of oxytocin in all four animals ($p<0.05$) and this was in accordance with suppressed uterine contractions. We conclude that morphine could be used as an inhibitor of nocturnal uterine contractions, and that this is caused by the morphine induced increased metabolic clearance rate of oxytocin.

Key Words: Morphine, oxytocin, metabolism, uterus, baboon

INTRODUCTION

Whether oxytocin (OT) clearance is increased during gestation has continued to be a subject of debate over the past years.[1] The majority of studies have focused on humans. However, initial investigations in women failed to find differences in the metabolic clearance rate (MCR) of OT during gestation.[2,3] More recently, Thornton et al.[1] reported a four- to five-fold increase in OT clearance in pregnant women. Humans appear to be unique in that OT and vasopressin in the blood may be degraded by oxytocinase, more appropriately referred to as cysteine aminopeptidase, a placental enzyme secreted into the blood.[4] The metabolism of OT occurs primarily in the kidneys, liver, and placenta.[5] Because MCRs of individual organs are additive, the MCR associated with OT would be expected to increase during gestation, supporting the report of Thornton et al.[1]

The physiological role of circulating OT is unequivocal during lactation because milk ejection reflex occurs.[6-8] In pregnancy, OT, along with other factors, is known to promote uterine contractions,[9] but its precise role in the initiation and progress of parturition is not fully understood. Over the past few years, morphine has been utilized as a tocolytic in humans and has been shown to transiently inhibit OT release.[10,11] The purpose of this study was to determine whether the tocolytic effect of morphine in the pregnant baboon is due to the enhanced MCR of OT, resulting in the suppression of uterine activity.

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Reprint address: requests to Dr. Sok Cheon Pak, Hamilton College of Oriental Medicine, PO Box 10, Hamilton, New Zealand. Tel: 64-7-838-0112, Fax: 64-7-838-0113, E-mail: sokcheonpak@hotmail.com

MATERIALS AND METHODS

Surgical procedure

Four pregnant baboons (15 - 18 kg) were ob-

tained from the breeding colony of the Biological Resource Laboratory at the University of Illinois at Chicago. The animals were kept under controlled lighting conditions (12 hours light/12 hours dark, lights on at 6 AM) and fed Purina Primate Chow (Ralston Purina, St. Louis, MO, USA). Normal delivery occurs at 184 ± 2 days. The protocol of this study was approved by the Animal Care Committee at the University of Illinois. Animals were adapted to the jacket for 1 week before surgery, which was performed at 140 - 150 days of gestation, for the insertion of three polyvinyl cannulas (0.86 × 1.32 mm) using a previously described procedure.[12] Briefly, under general anesthesia, a midline incision was made exposing the uterus. One cannula was inserted into the amniotic cavity using a 16-gauge needle as a trocar. A second incision was made in the groin area, and a similar cannula was inserted into the femoral vein and advanced into the inferior vena cava. A third cannula was inserted into the femoral artery and advanced to the aorta. All three cannulas were led subcutaneously to the scapula area and brought out through a small incision. A nylon mesh jacket (Alice King Chatham Medical Art, Los Angeles, CA, USA) was placed on the baboon, and the cannulas were brought out through a flexible stainless steel tether attached to the back of the jacket.

Measurement of uterine activity

An amniotic fluid cannula was attached to a P23id Gould pressure transducer, and intrauterine pressure changes were recorded on a Model 7 Grass Polygraph (both from Grass Instruments, Quincy, MA, USA). The vascular cannulas were attached to syringes located on a Harvard infusion pump (Holliston, MA, USA). Saline with heparin (100 U/mL) was infused at a rate of 0.582 mL/hr to keep the cannulas patent. When nocturnal uterine contractions were detected, either 5 mg of morphine sulfate in 5 ml of saline or 5 ml of saline only (control) was administered i.v. as a bolus infusion.

Oxytocin metabolism

To determine the effect of morphine on oxytocin metabolism, 4 baboons received an i.v. bolus infusion of 500 mU of oxytocin (Pitocin: Parke-Davis; 1 mU=2 pmol) at 13.00 h on 2 consecutive days. At 12.30 h, either morphine sulfate (5 mg) or saline was administered i.v. The order of treatment was randomized, and morphine preceded saline in two baboons. Two basal samples of arterial blood (3 ml) were collected before oxytocin injection at -10 min and -5 min, and blood was subsequently collected at 0.5, 1.5, 2.5, 5, 7.5, 10, 15, 20, 25, and 30 min. Blood sampling was performed between 170 and 175 days of pregnancy. Each blood sample (3 ml) was collected on ice, centrifuged at 2000 × g at 4℃ and the plasma was removed and frozen at -70℃ until used in the assay as described below.

Oxytocin radioimmunoassay

To measure plasma oxytocin by radioimmunoassay (RIA), we used a slight modification of the method described by Amico et al.[13] After extracting 0.5 ml plasma with petroleum ether/acetone, the solvent was evaporated and the residue reconstituted in 0.25 ml of buffer. Assay duplicates were incubated with the first oxytocin antibody for 48 hours at 4℃, ^{125}I-tyr^{2}-oxytocin was then added to each tube and incubation continued for another 18 hours. The second antibody was then added to each tube and the mixtures were incubated at room temperature for 20 minutes. Finally, all samples were analyzed for radioactivity in a gamma counter. The intra- and interassay coefficients of variation were 13% and 16%, respectively, and the assay sensitivity was 0.6 fmol/tube.

Oxytocin, for reference preparations, was purchased from Calbiochem (La Jolla, CA, USA), and ^{125}I-tyr^{2}-oxytocin was purchased from DuPont (Boston, MA, USA). The first oxytocin antibody was raised in sheep and was a gift from Dr. Flint (Institute of Zoology, The Zoological Society of London, UK). The cross-reactivity of this antiserum was less than 1% with oxytocin-glycine and less than 0.1% with arginine-vasopressin and lysine-vasopressin. Donkey anti-sheep IgG (Per-Septive Diagnostics Cambridge, MA, USA) was used as the second antibody to separate bound

and free ^{125}I-tyr^{2}-oxytocin. To determine the ratio of the extraction efficiencies and for monitoring the efficiency of oxytocin extraction in each sample, we used [tyrosyl-2,6-^{3}H]oxytocin (specific activity=48.5 Ci/mmol) and 8-L-arginine, [phenylalanyl-3,4,5-^{3}H(N)]vasopressin (specific activity= 81 Ci/mmol), which were purchased from DuPont (Boston, MA, USA).

The assay was validated for pregnant baboon samples by demonstrating the recovery of spiked samples and parallelism of sample dilutions to the standard curve. The recovery rate of the spiked samples was 102 ± 3%, and the sample dilutions followed the standard curve.

Data analysis

Uterine contractile activity was analyzed to determine the frequency, mean amplitude, and mean duration of the uterine contractions. Data for nocturnal uterine contractions were analyzed in three time blocks, i.e., -15 to 0, 0 to 15, and 30 to 45 min, where zero (0) min indicates the time of either morphine or saline infusion.

The disappearance of oxytocin from blood after a bolus dose was modeled by the weighted least squares method for two compartments using the PCNONLIN program (SCI Software, Lexington, KY, USA). The following OT metabolic parameters were calculated[14]: 1) the MCR of OT=OT dose/AUC_{OT}, where AUC_{OT}=area under the curve of OT concentrations vs. time from zero to infinity; 2) the volume of distribution at steady-state (V_{SS}) of OT=OT dose × $AUMC_{OT}/AUC_{OT}^{2}$, where $AUMC_{OT}$=area under curve of OT concentrations vs. time, extrapolated to infinity; and 3) the mean residence time (MRT) of OT, a statistical concept defined as the mean time that the material resides in the body, which was determined by the ratio of the V_{SS} and the MCR (e.g., MRT=V_{SS}/MCR). Half-lives for the two phases of OT disappearance, $t_{1/2\alpha}$ for the initial phase and $t_{1/2\beta}$ for the terminal phase, were estimated. The fraction of OT elimination associated with the terminal phase (f_b) was then calculated as $(0.693 \times C_b)/(t_{1/2\beta} \times AUC_{OT})$, where C_b=the zero-time intercept for the terminal exponential term. Data for MCR and V_{SS} were expressed per kilogram of body weight. For the statistical result, effects of morphine on the quantitative parameters of the OT metabolism were analyzed by paired t-tests. Results were considered significant when p was < 0.05. Data are expressed as means ± SE.

RESULTS

The effects of morphine or saline treatment on frequency, amplitude, and duration are summarized in Table 1. Overall, morphine treatment

Table 1. Effect of Morphine or Saline Administration on Three Parameters of Nocturnal Uterine Activity*

Parameter	Before treatment -15 to 0 min	After treatment	
		0 to 15 min	30 to 45 min
Frequency†			
Morphine	5.3 ± 0.3	3.2 ± 0.5§	1.4 ± 0.6§
Saline	4.8 ± 0.4	4.5 ± 0.6	4.6 ± 0.4
Mean amplitude‡			
Morphine	38 ± 5	27 ± 8§	23 ± 5§
Saline	35 ± 4	38 ± 6	40 ± 8
Mean duration			
Morphine	55 ± 2	51 ± 3	49 ± 5
Saline	53 ± 4	55 ± 2	57 ± 4

*Morphine (n=4); Saline (n=4); mean ± SE.
†Frequency/15 min.
‡Mean amplitude (mmHg).
§significantly different ($p<0.05$) from pretreatment interval.

significantly suppressed the frequency of uterine contractions ($p<0.05$) and the mean amplitude of the uterine contractions ($p<0.05$), but did not affect the duration of the uterine contractions ($p>0.05$). Both the frequency of the uterine contractions and their mean amplitude decreased immediately after morphine administration and decreased further during the subsequent 30 to 45 min interval.

The MCR of OT (ml/min/kg) was increased by about 40% from 18.0 ± 1.60 to 22.2 ± 1.60 (Table 2, $p<0.05$) by the infusion of 5 mg of morphine. This effect of morphine upon OT clearance was observed consistently in all 4 animals, and its magnitude ranged from 2% to 14% in individual animals. By contrast, morphine administration did not appear to consistently affect the V_{SS} of OT, which was 216 ± 31 ml/kg during control trials and 246 ± 33 ml/kg during trials with morphine (Table 2, $p>0.05$). The MRT of OT was 8.3 ± 0.8 min after saline administration and 8.8 ± 0.9 min after administering 5 mg of morphine (Table 2, $p>0.05$).

The initial half-life of OT was 1.1 ± 0.2 min for control trials with saline vs. 1.1 ± 0.3 min for trials with morphine (Table 2, $p>0.05$), and this was followed by a longer terminal half-life of 8.4 ± 0.8 min during control trials vs. 8.0 ± 0.9 min during morphine treatment (Table 2, $p>0.05$). The fraction of OT elimination associated with the terminal phase (f_b) was 0.65 ± 0.07% for trials with saline and 0.74 ± 0.04% for trials with morphine (Table 2, $p>0.05$). Overall, the equations of the two-compartment system fit the data for OT disappearance well, with correlation coefficients of 98-99%.

DISCUSSION

The present study found that morphine suppresses nocturnal uterine contractions by increasing the MCR of OT in the pregnant baboon. The increased clearance of OT might have been due to increased blood flow to the sites of OT elimination, such as the placenta, the kidneys, and the liver. Although such circulatory opiate effects have not been reported, they are plausible, because of interactions between circulatory opiate level and the vascular opiate receptor[15] or the release of vasoactive factors like histamine by morphine.[16] Other common causes that may lead to an increase in the MCR, such as increased enzyme activity in metabolizing organs (through the induction of enzyme synthesis) and a decreased protein binding in the circulation, are likely to have been features of our study, because of a rapid opiate effect on OT clearance, and because OT does not bind to plasma proteins.[17] There is a possibility that endogenous OT levels might interfere with MCR studies. However, MCR studies were performed at midday, a time when no uterine contractions occur and endogenous plasma OT levels are very low, as was confirmed

Table 2. Several Parameters of Oxytocin Disposition Determined with Either Control Saline or Morphine in the Pregnant Baboons

Parameter	Saline	Morphine	p
MCR	18.0 ± 1.60	22.2 ± 1.60	S
Vss	216 ± 31	246 ± 33	NS
MRT	8.3 ± 0.8	8.8 ± 0.9	NS
$t_{1/2\alpha}$	1.1 ± 0.2	1.1 ± 0.3	NS
$t_{1/2\beta}$	8.4 ± 0.8	8.0 ± 0.9	NS
f_b	0.65 ± 0.07	0.74 ± 0.04	NS

Data are mean ± SE. MCR (ml/min/kg), metabolic clearance rate; Vss (ml/kg), volume of distribution at steady-state; MRT (min), mean residence time; $t_{1/2\alpha}$ (min) and $t_{1/2\beta}$ (min), half-lives during the two phases (initial and terminal) of disappearance; f_b(%), fraction of elimination associated with terminal phase; p, level of statistical significance; S, significant; NS, not significant.

by a preliminary study (data not shown).

Data in the literature regarding the mode of OT secretion i.e., - continuous vs. pulsatile - are equivocal. Unlike lactation, during which OT release is unquestionably pulsatile,[6,8] gestational OT pulses have been documented in some,[18-20] but not in all[21] studies. Since the half life of OT in the pregnant baboon is very short (1.1 min), which is true for all species studied, including humans,[19] and because accurate blood sampling at frequencies higher than one minute was not feasible in our model, we limited the procedure to 1-min intervals, and therefore, did not analyze generated hormone profiles using any of the pulse detection algorithms. Since such parameters as $t_{1/2\alpha}$, $t_{1/2\beta}$, and f_b determine the time required to reach the steady-state;[14] therefore, one can predict what percentages of plateau concentrations have been reached at a given time.

In summary, our data indicate that in the pregnant baboon; 1) morphine suppresses nocturnal uterine contractions; and 2) OT clearance is increased by morphine. Furthermore, 3) OT clearance after bolus administration can be modeled using a two-compartmental system; and 4) the half-life of OT in the initial phase of the distribution is very short (1 min), and this is followed by a more prolonged terminal elimination half-life of - 8 min, but neither the initial nor the terminal phase half-lives are affected by morphine. The effect of morphine on uterine contractions at night is in part due to enhanced OT clearance, and indicates the tocolytic effect of morphine in the pregnant baboon.

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