Bile acids increase response and expression of human myometrial oxytocin receptor

Alfredo M. Germain, MD,[a,d] Sumie Kato, MSc,[a,d] Jorge A. Carvajal, MD, PhD,[a,d] Guillermo J. Valenzuela, MD, MBA,[e] Gloria L. Valdes, MD,[c,d] and Juan C. Glasinovic, MD[b]

Santiago, Chile, and Colton, Calif

OBJECTIVE: We tested the hypothesis that during intrahepatic cholestasis of pregnancy bile acids activate the myometrial oxytocin receptor pathway.

STUDY DESIGN: Myometrial sensitivity to oxytocin and oxytocin-receptor messenger RNA and protein level was investigated. The ability of cholic acid to mediate such changes was evaluated.

RESULTS: Cholestasis patients required lesser oxytocin to elicit four uterine contractions in 10 minutes (1.3 ± 0.6 vs 3.6 ± 0.8 U, $P < .05$, n = 7) and had lower in vitro ED_{50} (1.6×10^{-10} mol/L vs 1.0×10^{-8} mol/L, $P < .05$, n = 7) than controls. The 24-hour incubation of control myometrial strips (n = 7) with cholic acid (20 μmol/L) increased oxytocin sensitivity. Incubation of cultured myometrial cells (n = 5) with cholic acid increased oxytocin-receptor expression (messenger RNA and protein).

CONCLUSION: We demonstrate that during intrahepatic cholestasis of pregnancy, an activation of the oxytocin receptor pathway occurs. This event seems to be the result of a cholic acid–mediated increase in oxytocin-receptor expression. (Am J Obstet Gynecol 2003;189:577-82.)

Key words: Intrahepatic cholestasis of pregnancy, oxytocin receptor

Intrahepatic cholestasis of pregnancy (ICP) is a human pregnancy-specific liver disorder, which occurs in late gestation and is characterized by biochemical cholestasis and skin pruritus. The disease is harmless for the mother but not for the fetus. The reported risk of preterm delivery from descriptive and noncontrolled studies is as high as 44%[1] and the largest retrospective case-control study showed a 4-fold increase of spontaneous preterm delivery (odds ratio [OR] 3.98, 95% CI 1.96-8.22, $P < .05$).[2] Also, an association between cholestasis of pregnancy, fetal distress, and stillbirth has also been documented.[1,3]

In pregnant sheep, the acute intravenous administration of cholic acid induces preterm delivery in 20% of animals,[4] whereas chronic administration of cholic acid to the fetal sheep circulation induces uterine contractions and preterm delivery in 100% of the animals.[5] The nonpregnant rat myometrium shows a dose-related increase in myometrial responsiveness to oxytocin when incubated with cholic acid.[6] Finally, in humans, myometrium obtained from patients with ICP exhibited an exaggerated contractile response in vitro to pharmacologic doses (10^{-2} to 10^{-4} mol/L) of oxytocin compared with controls.[7]

We hypothesize that in ICP an activation of the oxytocin receptor occurs through a bile acid–mediated mechanism. To test this hypothesis, we compared myometrial response with oxytocin in vivo and in vitro in pregnancies complicated by cholestasis and controls. Further, in control samples, the effect of bile acids on oxytocin sensitivity and oxytocin-receptor expression in myometrial tissue and cells in culture was examined.

Material and methods

Patients. The study was conducted at the Pontificia Universidad Católica de Chile Medical School, during the years 1999 to 2001. The Institutional Review Board of our institution approved the study protocol. Patients were included in the protocols after giving their informed consent. The diagnostic criteria for ICP were those previously reported by us[2]: (1) continuous and generalized skin pruritus mainly located in palms and soles of nocturnal predominance, which disappears early in the puerperium, and (2) absence of clinical and ultrasonographic evidence of obstructive gallstone

From the Department of [a]Obstetrics and Gynecology, [b]Gastroenterology, [c]Nephrology, and [d]Medical Research Center, Pontificia Universidad Católica de Chile School of Medicine, Santiago, Chile, and the [e]Arrowhead Regional Medical Center, Colton, Calif, USA.

Supported by FONDECYT 1980964, DIPUC 97II/12 CE, DIPUC 3811102, and a Research and Development Grant from Gynopharm Laboratories I/98.

Received for publication October 25, 2002; revised March 25, 2003; accepted April 17, 2003.

Reprints not available from the authors.

0002-9378/2003 $30.00 + 0

doi:10.1067/S0002-9378(03)00545-3

disease, clinical evidence of viral hepatitis, fever or general malaise, or skin or medical disorders that could produce pruritus. The diagnosis of cholestasis was always made in a clinical basis; bile acids or other biochemical tests were not evaluated routinely. The patients' clinical management remained unchanged throughout the protocol and followed the guidelines of our institution.[2]

Oxytocin challenge test. Seven nulliparous cholestatic patients and seven control subjects matched by gestational age and Bishop score were evaluated. The oxytocin challenge test was performed as follows. At 38 weeks' gestation, patients were placed in a supine position in our labor/delivery unit. Fetal heart rate and uterine contractions were electronically monitored with external transducers. Baseline uterine contractions were recorded for 30 minutes and oxytocin was administered intravenously with a constant-infusion pump. The infusion started at 1 mU/min, with the rate being doubled every 30 minutes until a contraction frequency of four uterine contractions over a 10-minute interval was attained; the dose of oxytocin required to elicit this frequency was recorded. Drug administration was stopped after this end point was reached. After the test, one patient from the control group continued with uterine contractions and went into labor. The remaining patients were discharged home after 12 hours of observation. All patients were delivered at term, and all newborn infants were healthy.

Measurement of isometric tension in vitro. Myometrial samples were excised from patients who underwent elective cesarean section at term, not in labor for obstetric indications (eg, breech delivery). The tissue preparation was accomplished as follows. A small biopsy of myometrium was excised from the superior lip of the hysterotomy. Longitudinal strips of myometrial smooth muscle (6 × 1.5 mm) were cut and mounted for measurement of isometric force in water-jacketed muscle baths (Radnotti; Monrovia, Calif) containing oxygenated (95% oxygen/5% carbon dioxide) physiologic salt solution sodium chloride (120.5 mmol/L), potassium chloride (4.8 mmol/L), magnesium sulfate (1.2 mmol/L), sodium phosphate (1.2 mmol/L), sodium bicarbonate (20.4 mmol/L), calcium chloride (1.6 mmol/L), dextrose (10 mmol/L), and pyruvate (1 mmol/L), pH 7.4, at 37°C. The myometrial strips were stretched to the optimal length for maximal force development (L_0) by the application of 12*g* of force and conditioned by one contraction in physiologic salt solution that contained potassium chloride (60 mmol/L) substituted isotonically for sodium chloride. Test agents were assayed in physiologic salt solution. The contractile response was quantified as the area under the curve of the contraction tracing per tissue cross-sectional area at 10-minute intervals. The cross-sectional area of each tissue was determined by using the following formula: $W/(L \times D)$, where W is the wet weight of tissue in grams, L is the stretched length in centimeters, and D is tissue density (1.05 g/cm^3).[9]

This preparation was used to perform a cumulative dose-response to oxytocin (10^{-12} to 10^{-7} mol/L) with strips from ICP (n = 7) and control (n = 7) patients obtained at 38 weeks' gestation, not in labor. In addition, strips of term myometrium obtained from patients with normal pregnancies were used to test the effects of long-term (24-hour) cholic acid (sodium salt; 20 μmol/L) incubation on the uterine response to oxytocin, endothelin-1 and prostaglandin $F_{2\alpha}$ (10^{-12} to 10^{-7} mol/L). Seven strips were used for each control or treated condition. Preliminary studies from our laboratory indicate that the myometrium retained normal oxytocin responsiveness until approximately 48 hours after the sample was obtained. Samples used for these experiments are from patients not enrolled for any other part of this study.

Myometrial cells in culture. Myometrial smooth muscle cells were isolated from myometrial tissue of healthy pregnant women obtained from patients undergoing elective cesarean section at term, not in labor. Briefly, the myometrial tissue was minced and incubated in a water bath with agitation for 4 hours at 37°C with collagenase (1.5 mg/mL, ~150 U/mg), deoxyribonuclease I (0.1 ng/mL, ~2000 U/mg), and antibiotic-antimycotic solution (2%, vol/vol) to disperse the smooth muscle cells. The dispersed cells were separated from nondigested tissue by filtration through gauze and collected by centrifugation of the filtrate at 400*g* for 10 minutes; suspended in Ham's F-12: Dulbecco's modified Eagle's medium (F12/DMEM, 1:1, vol/vol) with fetal bovine serum (FBS, 10%, vol/vik), penicillin G (100 U/mL), streptomycin sulfate (100 μg/mL), and amphotericin B (0.25 μg/mL); plated in plastic culture dishes (100 mm diameter); and maintained at 37°C in a humidified atmosphere of air and carbon dioxide (5%) until confluence (7-10 days after plating).[10] The culture medium was changed every 48 hours. After confluence, the culture medium was changed; and 24 hours thereafter, the cells were incubated for 24 hours in serum-free F12/DMEM before the medium was changed to serum-free medium that contained the test agents.

Western blot of oxytocin receptor. Myometrial cells were washed with phosphate-buffered saline (PBS), and harvested with phenylmethylsulfonylfluoride solution (PMSF), then frozen in liquid nitrogen and stored at −80°C. The pellet was suspended in buffer (0.1 mol/L sodium chloride, 0.01 mol/L TRIS Cl, pH 7.6, 0.001 mol/L EDTA pH 8, 1 μg/mL aprotinin, 100 μg/mL PMSF). Cells were disrupted by sonication and centrifuged 5 minutes at 1000*g*. Pellets were suspended in suspension buffer and the protein concentration determined by Bradford analysis.[11] Proteins (10 μg per lane) were separated by SDS-PAGE on 8% minigel and electrotransferred to nitrocellulose membranes (1 hour at 100 v).

Blots were blocked overnight in PBS containing 5% nonfat milk, then washed with PBS 0.05% Tween 20, and incubated with a specific monoclonal antibody previously characterized.[12] Antihuman oxytocin-receptor antibody (O-2F8) as primary antibody (gently provided by ROHTO Pharmaceuticals Co, LTD, Osaka, Japan) at a final concentration of 1.4 μg/mL. After incubation, the blots were washed (PBS 0.05% Tween 20) and incubated with peroxidase-labeled antimouse antibody (1:2000). Blots were again washed and immunoreactive bands were visualized by using Amersham ECL (enhanced chemiluminescence) system.

Inmunohistochemistry of oxytocin receptor. The cell samples were fixed in cold acetone (−20°C) for 10 minutes. Thereafter, they were washed three times, 5 minutes each, with buffer (PBS) and incubated with O-2F8 primary antibody (1:100) for 18 hours, followed by three PBS washes. Then, samples were incubated with IgG rabbit antimouse Dako Z0456 (1:50) for 30 minutes and then samples were washed and incubated with IgG swine antirabbit Dako Z0196 (1:100) for 30 minutes, washed again and incubated with Pap rabbit Dako Z0113 (1:100) for 30 minutes.

The number of myometrial cells that expressed positive reactivity for the oxytocin receptor were evaluated in four opposing fields of confluent cells at 400× final magnification. Each field was graded 0 to 3, according to the amount of cells stained for the oxytocin receptor, with 0 = no cells stained, 1 = scant cells stained, 2 = moderate amount of cells stained, and 3 = most cells stained. The final grading was represented by the sum of grades of each field. This grading was tested both by counting stained cells per field, and by two independent observers, blinded to the experimental group of the samples; the correlation between the grading of each observer was $r = 0.89$. In addition, the κ agreement index was 0.65.

Northern blot of oxytocin receptor. Total RNA extraction and northern blot procedure were performed as described.[13] Briefly, frozen tissues were pulverized in liquid nitrogen with a cold mortar and pestle. The tissue powder was dissolved in guanidinium isothiocyanate (4 mol/L); the mixture was layered over a solution of cesium chloride (5.7 mol/L) and centrifuged at 238,000*g* for 18 hours at 25°C. The RNA pellet was suspended in sodium acetate (0.3 mol/L, pH 6.0) and precipitated by the addition of ethanol at −80°C. Total RNA (5-20 μg per lane), was size fractionated by electrophoresis on formaldehyde-agarose (1%) gels and transferred electrophoretically to nylon membrane (Hybond-N^+; Amersham, Arlington Heights, Ill). The membranes were heated in a vacuum at 80°C for 60 minutes. For complementary DNA (cDNA) probes, prehybridization was conducted by 16 hours at 43°C in a solution of formamide (50%, vol/vol), sodium phosphate (50 mmol/L, pH 7.2), 10× Denhardt solution, 5× SSC, dextran sulfate (5%, wt/vol), and salmon sperm DNA (0.5 mg/mL). Hybridization was conducted for 16 hours at 43°C in a solution of formamide (50%, vol/vol), sodium phosphate (20 mmol/L, pH 7.2), 2× Denhardt solution, 5× SSC, dextran sulfate (10%, wt/vol), and salmon sperm DNA (0.25 mg/mL) with a specific cDNA probe. The DNA probe used for oxytocin receptor, was synthesized from a 0.81-kb fragment of the human oxytocin receptor gene.[14] The cDNAs were radiolabeled with [α-^{32}P]dCTP random hexamer priming. After hybridization, the blots were shed with 2× SSC and SDS (0.1%, wt/vol) for 15 minutes at 25°C, twice with 0.1× SSC and SDS (0.1%, wt/vol) for 20 minutes at 25°C, and twice for 15 minutes at 43°C. The amount of total RNA in each lane was evaluated by visualization of 28S and 18S ribosomal RNA subunits.

Autoradiography of the membranes was performed at −80°C by using Kodak X-Omat AR film. The bands we analyzed with densitometric analysis of the specific oxytocin-receptor transcript by using a densitometer coupled to analytic software (Scientific image system, Kodak 1.0 2D). Results were normalized by the 18S ribosomal RNA subunits band on each lane.

Reactives. All reagents used were from Sigma (St Louis, Mo), unless specifically stated.

Statistical analysis. The in vitro oxytocin myometrial sensitivity and cholic acid effect on oxytocin-receptor expression at mRNA and protein levels (immunohistochemistry) were analyzed by analysis of variance (ANOVA) and Newman-Keuls test. The agreements between the observers for the inmunohystochemistry staining were evaluated by the κ coefficients. The data from in vivo oxytocin sensitivity and cholic acid effect of oxytocin-receptor protein levels (Western blot) were compared by Student *t* test. The data are presented in the text as the mean ± SEM. A two-tailed $P < .05$ was considered indicative of statistically significant difference.

Results

Oxytocin challenge test. Patients with ICP, paired with controls by using Bishop score (4.8 ± 1.0 and 4.6 ± 0.7, respectively) and gestational age (38 ± 0.2 and 38.2 ± 0.1, respectively) required a significantly lower minimal effective dose of oxytocin to elicit a frequency of four uterine contractions in a 10-minute period (1.3 ± 0.6 vs 3.6 ± 0.8 U, $P < .05$, Fig 1).

In vitro dose-response curve to oxytocin. A significantly higher basal and dose-related increase in myometrial contractile responsiveness to oxytocin was observed in strips from cholestatic patients compared with controls ($P < .05$, Fig 2). The effective dose 50 (ED_{50}) was 1.6×10^{-10} mol/L in cholestatic and 1.0×10^{-8} mol/L in controls ($P < .05$).

Effects of cholic acid incubation on in vitro myometrial contractile response. The 24-hour incubation of control

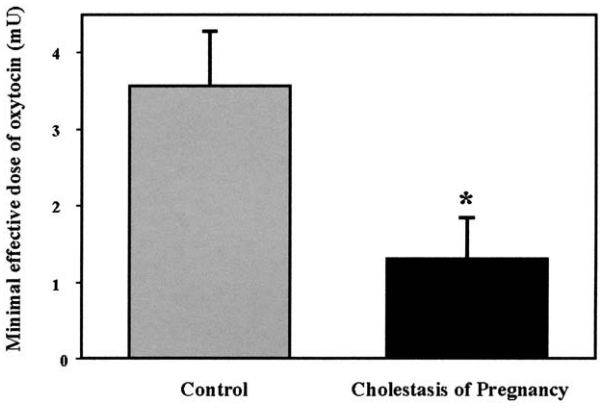

Fig 1. Oxytocin challenge test. Minimal effective dose of oxytocin necessary to achieve four uterine contractions in 10-minute period. Data correspond to mean ± SE from seven cholestatic and seven control patients. *Asterisk, P* < .05.

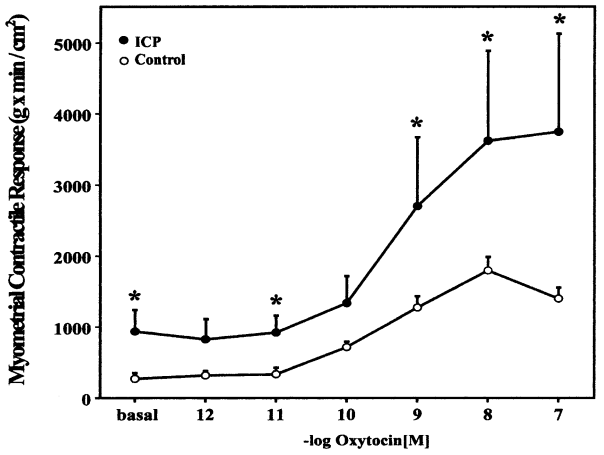

Fig 2. Dose-response curve to oxytocin. Measurement of isometric tension in vitro using human term myometrium from control (n = 7) and ICP (n = 7) patients. Data presented as mean ± SE. *Asterisk, P* < .05.

myometrial samples with cholic acid (20 μmol/L) resulted in an increased contractile response to oxytocin compared with incubation with vehicle ($P < .05$, Fig 3). This effect was specific for oxytocin because no effects on the myometrial response to endothelin-1 or prostaglandin $F_{2\alpha}$ were observed. In addition, cholic acid (10^{-7} to 10^{-3} mol/L, n = 4 experiments) by itself did not alter myometrial contractility.

Effect of cholic acid incubation on oxytocin receptor mRNA levels. The 24-hour incubation of cultured myometrial cells with cholic acid (0 to 1000 μmol/L) produced a dose-related increase in oxytocin-receptor mRNA levels (n = 5, $P < .05$, Fig 4). The time-curve (0, 6, 12, 24, and 48 hours) demonstrates that the cholic acid effect started at 6 hours with a maximal effect at 24 hours of incubation. The cholic acid effect seems to be specific

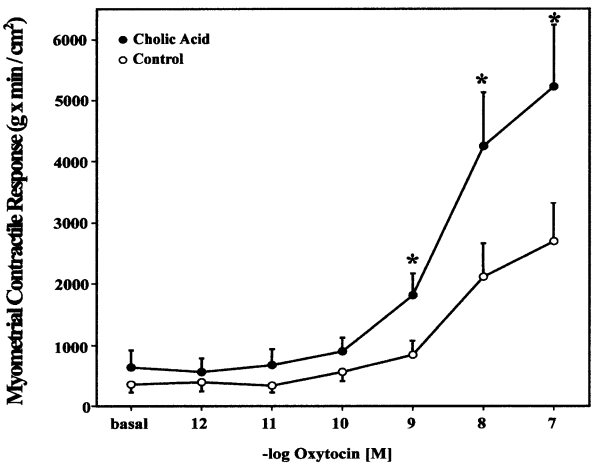

Fig. 3. Dose-response curve to oxytocin in myometrium treated with cholic acid. Myometrial strips were incubated during 24 hours in control (n = 7) or cholic acid 20 μmol/L conditions (n = 7). Data presented as mean ± SE. *Asterisk, P* < .05.

because a separate series of experiments using cholesterol (n = 3, dose ranging from 2 to 1000 μmol/L) or deoxycholic acid (n = 3, dose ranging from 0.2 to 200 μmol/L) did not induce changes in mRNA levels of the receptor.

Effects of cholic acid incubation on oxytocin receptor protein expression. In addition to the increased mRNA levels, cholic acid increased the receptor at the protein level as evidenced by both Western blot (n = 5, $P < .05$, Fig 5) and immunohistochemistry (Fig 6). In addition to the main band of oxytocin receptor protein, a second band was observed. The analysis suggested a band of nonspecific nature because it was not observed in all blots. The semiquantitave evaluation of the immunohistochemistry revealed that there was a dose response of protein levels to cholic acid incubation: 3.0 ± 0.9 (control), 5.5 ± 0.3 (2 μmol/L), 8.3 ± 0.9 (20 μmol/L), 9.3 ± 1.1 (200 μmol/L), and 5.0 ± 1.9 (1000 μmol/L) (n = 5, $P < .05$, Fig 6).

Comment

The current results demonstrate enhanced myometrial oxytocin sensitivity, both in vivo and in vitro, in patients with ICP (or tissues) compared with controls. In addition, our data indicate that cholic acid acts on the myometrium to increase oxytocin sensitivity and to increase the expression of the oxytocin-receptor at mRNA and protein level. These findings provide a possible mechanism to explain the increased incidence of preterm delivery observed in patients with cholestasis of pregnancy.

We hypothesize that in the setting of ICP, the oxytocin receptor pathway is activated prematurely in those patients with cholestasis-related preterm labor. In support of this hypothesis, this study demonstrated enhanced

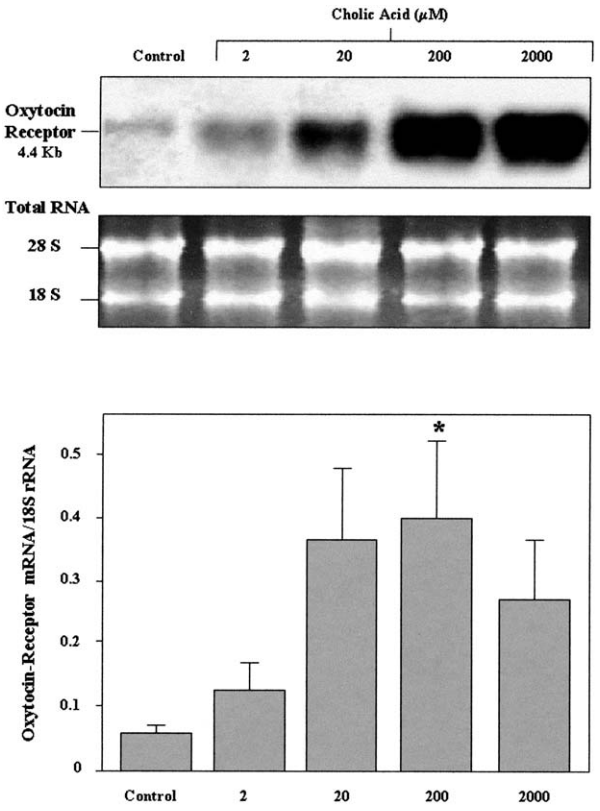

Fig 4. Northern blot for oxytocin receptor mRNA on cultured myometrial cells. Cells were incubated with increasing concentrations of cholic acid (n = 5). *Asterisk,* $P < .05$. In the bottom mean ± SE of the five experiments.

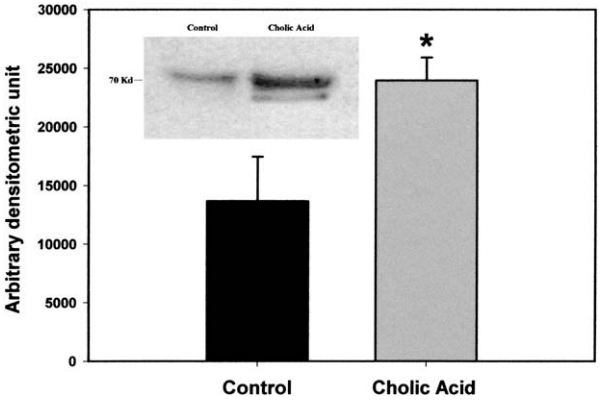

Fig 5. Densitometric analysis of Western blots of oxytocin receptor on myometrial samples after 24-hour exposure to 20 μmol/L cholic acid (n = 5). Data presented and mean ± SE. *Asterisk,* $P < .05$. In the insert a representative blot shows a myometrial sample incubated with cholic acid.

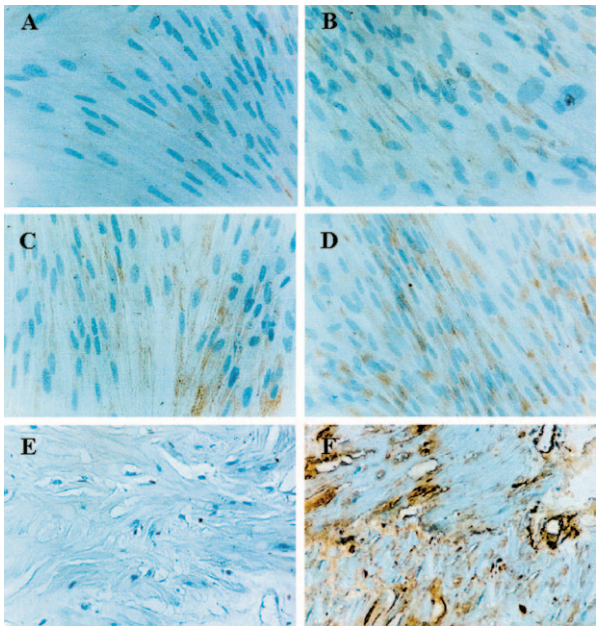

Fig 6. Immunohistochemistry of oxytocin receptor on cultured myometrial cells. **A**, Control. **B**, 2 μmol/L cholic acid. **C**, 20 μmol/L cholic acid. **D**, 200 μmol/L cholic acid. **E**, Negative control (myometrial sample without primary antibody). **F**, Positive control (term myometrium). (Stain, peroxidase anti-peroxidase; original magnification, ×400).

myometrial sensitivity to oxytocin in term cholestatic patients, using both in vivo and in vitro studies.

We observed that the long-term incubation of myometrial strips with cholic acid, at a concentration similar to that observed in the plasma of patients with cholestasis, led the myometrium to develop a contractile phenotype (eg, a high-oxytocin sensitivity) similar to that observed in patients with ICP. The effect seems to be specific to oxytocin because no change in myometrial sensitivity to endothelin-1 or prostaglandin $F_{2\alpha}$ was observed. These data support the hypothesis that cholic acid may mediate the enhanced myometrial sensitivity to oxytocin, observed in patients or tissues with ICP.

In addition, the treatment of myometrial cells with cholic acid elicited a dose-related increase in the expression of oxytocin receptor mRNA and protein. The specificity of cholic acid effect was confirmed because cholesterol or deoxycholic acid did not increase gene expression of the receptor. These data provide a possible molecular explanation for the increased response to oxytocin observed after cholic acid incubation.

In conclusion, we have found: greater in vivo and in vitro response to oxytocin during ICP; enhanced myometrial sensitivity to oxytocin after incubation with cholic acid; and up-regulation of oxytocin receptor expression (mRNA and protein) after exposure to cholic acid. Our results describe a potential in vivo role of bile acids in mediating an increased sensitivity to oxytocin. This increased sensitivity to oxytocin may in turn explain the greater incidence of preterm labor observed in patients whose pregnancies are complicated by intrahepatic cholestasis.

We thank all the medical and paramedical staff of our labor and delivery unit for the gracious support in obtaining the myometrial tissues and Mrs Cecilia Chacon and Rossana Vidal for their invaluable technical support.

REFERENCES

1. Fisk NM, Storey GN. Fetal outcome in obstetric cholestasis. BJOG 1988;95:1137–43.
2. Rioseco AJ, Ivankovic MB, Manzur A, Hamed F, Kato SR, Raer JT, et al. Intrahepatic cholestasis of pregnancy: a retrospective case-control study of perinatal outcome. Am J Obstet Gynecol 1994; 170:890–5.
3. Johnston WG, Baskett TF. Obstetric cholestasis: a 14 year review. Am J Obstet Gynecol 1979;133:299–301.
4. Perez R, Garcia M, Ulloa N, Jara C, Bardisa L, Rudolph MI. A single intravenous high dose of cholic acid to a pregnant ewe does not affect fetal well-being. Res Exp Med (Berl) 1994;194:63–7.
5. Campos GA, Guerra FA, Israel EJ. Effects of cholic acid infusion in fetal lambs. Acta Obstet Gynecol Scand 1986;65:23–6.
6. Campos GA, Castillo RJ, Toro FG. Effect of bile acids on the myometral contractility of the isolated pregnant uterus. Rev Chil Obstet Ginecol 1988;53:229–33.
7. Israel EJ, Guzman ML, Campos GA. Maximal response to oxytocin of the isolated myometrium from pregnant patients with intrahepatic cholestasis. Acta Obstet Gynecol Scand 1986;65:581–2.
8. Germain AM, Valenzuela GJ, Ivankovic M, Ducsay CA, Gabella C, Seron-Ferre M. Relationship of circadian rhythms of uterine activity with term and preterm delivery. Am J Obstet Gynecol 1993; 168:1271–7.
9. Word RA, Kamm KE, Casey ML. Contractile effects of prostaglandins, oxytocin, and endothelin-1 in human myometrium in vitro: refractoriness of myometrial tissue of pregnant women to prostaglandins E2 and F2 alpha. J Clin Endocrinol Metab 1992;75:1027–32.
10. Casey ML, MacDonald PC, Mitchell MD, Snyder JM. Maintenance and characterization of human myometrial smooth muscle cells in monolayer culture. In Vitro 1984;20:396–403.
11. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
12. Takemura M, Kimura T, Nomura S, Makino Y, Ioneu T, Kikuchi T, et al. Expression and localization of human oxytocin receptor mRNA and its protein in chorion and decidua during parturition. J Clin Invest 1994;93:2319–23.
13. Germain AM, MacDonald PC, Casey ML. Endothelin receptor mRNAs in human fetal membranes, chorionic vessels, and decidua parietalis. Mol Cell Endocrinol 1997;132:161–8.
14. Kimura T, Tanizawa O, Mori K, Brownstein MJ, Okayama H. Structure and expression of a human oxytocin receptor. Nature 1992; 356:526–9.
15. Glasinovic JC, Marinovic I, Vela P, Lopez J, Ahumada E, Valdiva MT, et al. Variations in serum bile acid levels in cholestasis of pregnancy. Rev Med Chil 1982;110:640–3.
16. Heikkinen J, Maentausta O, Ylostalo P, Janne O. Changes in serum bile acid concentrations during normal pregnancy, in patients with intrahepatic cholestasis of pregnancy and in pregnant women with itching. BJOG 1981;88:240–5.
17. Reyes H. The enigma of intrahepatic cholestasis of pregnancy: lessons from Chile. Hepatology 1982;2:87–96.
18. Phaneuf S, Asboth G, Carrasco MP, Euope-Finner GN, Saji F, Kimura T, et al. The desensitization of oxytocin receptors in human myometrial cells is accompanied by down-regulation of oxytocin receptor messenger, RNA. J Endocrinol 1997;154:7–18.
19. Seron-Ferre M, Taylor NF, Martin MC, Leake RD. Development of a circadian variation of plasma oxytocin concentration in the late gestation rhesus monkey. J Clin Endocrinol Metab 1991; 72:1323–7.
20. Ducsay CA, Cook MJ, Walsh SW, Novy MJ. Circadian patterns and dexamethasone-induced changes in uterine activity in pregnant rhesus monkeys. Am J Obstet Gynecol 1983;145:389–96.