pyramidal cells (*20*). It remains to be determined whether all or part of the after-hyperpolarization seen in the LC is also due to an increase in Ca^{2+}-dependent G_K. Such a finding would raise the possibility that α_2-adrenoceptors operate through a Ca^{2+}-dependent mechanism to hyperpolarize LC cells, as has been suggested for sympathetic neurons (*18*).

Intracellular recordings from LC neurons in a brain slice preparation have shown that opiates and opioid peptides produce a naloxone-reversible hyperpolarization of membrane potential associated with an increase in membrane conductance (*21*). These opiate-induced membrane effects resemble those we have observed with clonidine. Despite these similarities, clonidine and the opiates have been shown to act at different receptors in the LC (*22*). Nevertheless, it is possible that α_2-agonists and opiates hyperpolarize LC neurons through a common final mechanism (such as an increase in G_K). Such similarities between the effects of α_2-agonists and opiates on LC neurons may provide a basis for the proposal that clonidine suppresses symptoms of opiate withdrawal by a functionally parallel action on central noradrenergic neurons (*22, 23*).

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14. To increase the stability of recording, a cluster of four No. 0 insect pins (Clay Adams) (set 2 mm apart in a square pattern and held in a slotted plate) was placed vertically into the brain surrounding the recording site. The pins seemed to improve stability by damping the transmission of cardiac and pulmonary pulsations within the brain near the recording site: high-gain d-c recordings showed a dramatic decrease in pulsatile potentials after placement of the pins. Pulsations were also reduced if the membrane over the cisterna magna was punctured to allow for drainage of cerebrospinal fluid. Stability was also improved in some cases if body temperature was allowed to drop several degrees below 36°C; no effect of lowered body temperature was observed on LC cell properties. Animals were also given O_2 by nasal tube to prevent labored respiration.
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Oxytocin Receptors and Human Parturition: A Dual Role for Oxytocin in the Initiation of Labor

Abstract. *The concentration of oxytocin receptors increased in the myometrium of pregnant women and reached maximum levels in early labor. Concentrations of oxytocin receptors were also high in the decidua and reached a maximum at parturition. In vitro, prostaglandin production by the decidua, but not by the myometrium, was increased by the addition of oxytocin. Oxytocin may therefore stimulate uterine contractions by acting both directly on the myometrium and indirectly on decidual prostaglandin production. Oxytocin receptors are probably crucial for the onset of human labor, and the stimulus for the increase in uterine prostaglandins may be oxytocin originating from the fetus.*

The mechanism of the initiation of human parturition remains an enigma. The concentrations of estrogen and progesterone, the main regulatory hormones in the maternal circulation, do not appear to change at the onset of parturition (*1*). Activation of the fetal adrenals, an important factor in the onset of parturition in sheep and goat, does not seem to be of critical importance for the timing of parturition in the human. Oxytocin and prostaglandins, potent stimulators of uterine contractions, are secreted during human parturition (*2*), but whether their concentrations in the maternal circulation increase as a cause or as a consequence of uterine contractions is not known; nor has a stimulus been detected for the increased production of prostaglandins during labor.

The absence in pregnant women of any of the clear and consistent changes in the concentrations of humoral factors that are associated with parturition in many animal species prompted us to search for changes at the tissue level. Soloff *et al.* (*3*) demonstrated that myometrial oxytocin receptor concentrations in pregnant rats increased shortly before parturition and reached maximum levels at delivery. Our purpose in the present study was to measure the concentration of oxytocin receptors in the uterus of pregnant women and to determine whether this concentration increases at the time of parturition. The discovery, in the course of this investigation, of high levels of oxytocin receptors in uterine decidua prompted a further study of the role of these receptors in uterine physiology.

Samples of myometrium and decidua parietalis were obtained from women delivering by cesarean sections before or at term. Samples of myometrium and endometrium were also obtained from the uteri of nonpregnant women undergoing hysterectomy. All tissues were placed on ice and transported to the laboratory for storage at −85°C until assayed. The oxytocin receptor concentrations were measured in a crude membrane fraction of myometrial and decidual homogenates (pellet sedimenting between 10,000*g* and 100,000*g*) as described (*4*), with [^{3}H]tyrosine-oxytocin being used as the radioactive ligand. The buffer used for homogenization contained 1 m*M* EDTA to dissociate endogenous oxytocin from its binding sites. This dissociation permitted us to determine the total number of receptor sites when the samples were exposed to endogenous oxytocin in vivo (*4*). Scatchard analyses were performed with increasing concentrations of unlabeled oxytocin. Nonspecific binding was measured by the addition of 0.2 μ*M* unlabeled oxytocin. In many instances, a single point assay was performed in duplicate with a subsaturating concentration of [^{3}H]oxytocin (0.6 n*M*). This concentration of [^{3}H]oxytocin was used to minimize nonspecific binding, which was about 20

percent of the maximum amount of oxytocin bound. Because Scatchard analyses indicated that the affinity of receptor sites for oxytocin was uniform in separate portions of the uterus and throughout gestation (apparent affinity constant, K_d, was 1 nM to 2 nM) (Table 1), changes in the binding of a subsaturating concentration are proportional to the changes in the total number of oxytocin receptors. The results are therefore expressed as relative amounts of oxytocin bound per milligram of DNA.

During gestation, the myometrial receptor concentrations increased from 27.6 ± 7.9 fmole per milligram of DNA in the uteri of nonpregnant women to 171.6 ± 67.4 fmole/mg in uteri at midgestation and to 1391 ± 180 fmole per milligram of DNA at term. Maximum receptor concentrations were found in the myometrium during early labor at term: 3583 ± 857 fmole per milligram of DNA. During preterm labor, the myometrial receptor concentrations (2343 ± 316 fmole per milligram of DNA) were also higher than the concentrations in normal pregnant women at term but not in labor (Table 1). The binding affinity to oxytocin did not change during gestation (Table 1), confirming the results of Sakamoto *et al.* (*5*).

The increase in oxytocin receptor concentrations was correlated with the changes in uterine sensitivity to oxytocin. The threshold dose to produce contractions decreases from about 500 to 1000 mU in nonpregnant women to 10 to 25 mU in pregnant women at term (*6*). Before term, the uterine sensitivity to oxytocin is greater in women who later deliver prematurely than in women with normal pregnancies at the same gestational age; at delivery the uterine oxytocin sensitivity is equally high in preterm and term parturients (*7*).

The oxytocin receptor concentrations were low in samples obtained in advanced labor. We ascribe this to the fact that during labor the cervix and lower uterine segment are pulled up with the result that the incision is now made through tissue which is anatomically different from that incised before or in early labor. In samples obtained at the upper end of a longitudinal section in advanced labor, the receptor concentration was 2604 ± 143 fmole per milligram of DNA (N = 3), whereas at the lower end the concentration was 501 ± 65 fmole (N = 3), similar to the values from transverse incision in advanced labor and thus supporting our contention.

We have shown previously in rats that uterine sensitivity and the magnitude of the uterine response to oxytocin are directly related to the concentration of uterine oxytocin receptors (*8*). The increased concentration of receptors, therefore, allows for an increase in the concentration of the oxytocin-receptor complex in the face of the relatively low concentrations of circulating oxytocin encountered during early stages of labor [2×10^{-11} to $1 \times 10^{-10} M$ (*2*)]. The elicitation of hormone response with only partial receptor occupancy has been shown with a number of systems, including neurohypophyseal hormones (*9*).

An unexpected finding was a high concentration of oxytocin receptors in the parietal decidua, where the levels were maximum in preterm labor and in early term labor (Table 1). The decidual receptors had the same affinity for oxytocin as the myometrial receptors; the apparent K_d was $1.63 \pm 0.063 \times 10^{-9} M$ (N = 8). Decidua possesses high levels of prostaglandin synthetase activity (*10*), and endometrial and uterine prostaglandin synthesis is stimulated by oxytocin in sheep and rat (*11*). We considered the possibility that the oxytocin binding sites in the decidua mediate the increase in prostaglandin synthesis, and therefore measured the influence of oxytocin on the production of prostaglandins E and F by decidual and myometrial tissues in vitro. Oxytocin increased the prostaglandin production in decidua but did not influence myometrial prostaglandin production (Fig. 1). Moreover, the basal and oxytocin-stimulated prostaglandin productions in the decidua were significantly higher in samples taken after the onset of labor than before labor.

Plasma oxytocin concentrations were measured by radioimmunoassay (*12*) in

Table 1. Oxytocin receptors in samples of human uteri obtained at low flap cesarean sections (transverse incision). The values (means ± standard error) are expressed as femtomoles of oxytocin bound per milligram of DNA. Values in each vertical row with different superscripts are significantly different; Student's t-test, $P < .05$.

Group	N	Myometrium	N	K_d (nM)	N	Decidua	N	K_d (nM)
Nonpregnant, menstruating	14	$27.6^{a} \pm 7.97$	4	2.7 ± 0.49	6	$24.9^{a*} \pm 5.59$		
Pregnant (13 to 17 weeks)	5	$171.6^{b} \pm 67.4$	1	1.85	1	629^{b}	1	1.4
Preterm labor (28 to 36 weeks)	8	$2353^{c} \pm 358$	6	1.63 ± 0.22	8	$3673^{c} \pm 947$	4	1.75 ± 0.23
Before labor (37 to 43 weeks)	6	$1391^{d} \pm 180$	6	1.65 ± 0.74	6	$1510^{d} \pm 382$	2	2.25 ± 0.35
Early labor† (37 to 43 weeks)	5	$3468^{c} \pm 886$	5	1.44 ± 0.12	3	$3177^{c} \pm 1426$		
Advanced labor‡ (37 to 43 weeks)	7	$257^{b} \pm 104$	3	2.03 ± 0.7	2	$786^{b,d}$	1	2.0

*Endometrium. †Patients scheduled for cesarean section when labor began. ‡Emergency cesarean sections.

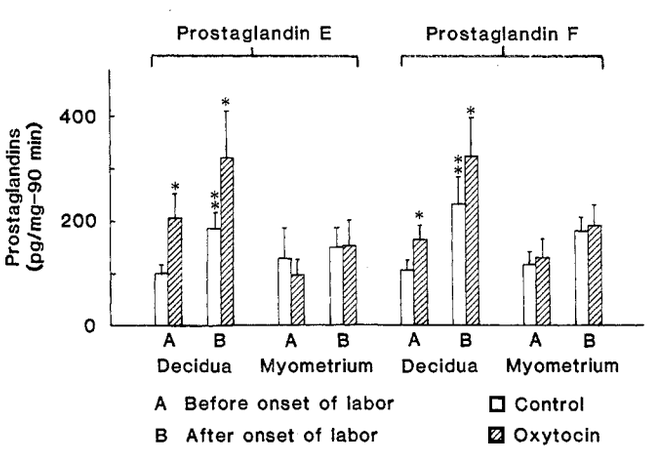

Fig. 1. Influence of oxytocin on decidual and myometrial prostaglandin production in vitro. Tissues were placed in ice-cold Krebs-Ringer solution containing a prostaglandin synthetase inhibitor (Amfenac, $1.4 \times 10^{-5} M$) and transported to the laboratory. They were then rinsed and incubated in regular Krebs-Ringer containing glucose, pH 7.4, at 35°C, under an atmosphere of 95 percent O_2 and 5 percent CO_2 with or without 10 mU of oxytocin per milliliter. After 30 minutes the medium was removed for assay and fresh medium was added. Prostaglandins were extracted from the acidified medium (pH 4.5) with a mixture of ethyl acetate and cyclohexane (1:1 by volume); portions of the dried extracts were assayed for prostaglandins E and F by means of specific radioimmunoassays (*15*).

maternal blood taken shortly before surgery and in umbilical arterial and venous blood taken at delivery (five to eight samples were obtained for each group). Before the onset of labor, levels were low in maternal (27.6 ± 10 pg/ml) and umbilical arterial blood (11.8 ± 6.2 pg/ml). In very early labor, the maternal level was unchanged (22.7 ± 6.3 pg/ml) but the umbilical arterial level significantly elevated (36.1 ± 11.7 pg/ml). In advanced labor both maternal (45.2 ± 17 pg/ml) and umbilical arterial oxytocin levels (57.3 ± 16 pg/ml) were higher than before labor.

From these results we propose that the following sequence of events results in the initiation of labor. The concentration of oxytocin receptors increases dramatically during gestation, probably under the influence of the rising estrogen levels. Evidence for the stimulation of oxytocin receptor formation by estrogens has been found in rabbits (*13*) and rats (*4, 8*). Near term, the rapid fetal growth rate accelerates uterine distension which probably contributes to the increase in oxytocin receptors toward term, as shown in rats (*14*). Although there is no dramatic increase in circulating oxytocin at the onset of labor, the increasing concentration of receptors lowers the oxytocin threshold to the point where activation of the myometrium occurs. Simultaneously, oxytocin binds to the receptors in the decidua, stimulating prostaglandin synthesis. The released prostaglandins diffuse into the adjacent myometrium and enhance the oxytocin-induced contractions. We have shown that oxytocin-induced contractions will not dilate the cervix and lead to progressive labor unless there is simultaneous prostaglandin release (*15*). The coupling of the oxytocin receptor activation and prostaglandin synthetase activity in the decidua therefore appears to be a crucial event in the initiation of labor. Additional support for this concept is provided by the fact that both ethanol, which inhibits oxytocin release, and prostaglandin synthetase inhibitors like indomethacin, inhibit labor contractions and can be used to prevent preterm birth (*16*).

We have confirmed the fetal secretion of oxytocin at term and the arteriovenous difference in oxytocin concentrations in the umbilical cord. Although experimental limitations make it difficult to document a transfer of fetal oxytocin through the human placenta and fetal membranes, such transfer has been shown in guinea pig and baboon (*17*). The amniotic fluid at term contains considerable amounts of oxytocin (*12*), and morphological studies of the human fetal membranes at term support the view that the circulating amniotic fluid, after traversing the amnion, will continue through the intercellular canaliculi of the chorionic cytotrophoblast to reach the decidua parietalis (*18*). We therefore postulate that fetal oxytocin can provide a stimulus for the increased production of prostaglandins at the onset of labor.

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Rapid Electronic Autofluorography of Labeled Macromolecules on Two-Dimensional Gels

Abstract. *The feasibility of electronically locating and measuring tritium-labeled macromolecules directly on dried electrophoretic gels has been demonstrated. This new procedure eliminates the usual long film exposure in autofluorography and the attendant delay in processing and data reduction. An image intensifier and electronic camera tube are used to integrate the light produced by the tritium interaction with a scintillator incorporated in the gel. Preliminary results show that, compared to film, the exposure is reduced 100 to 1000 times. The response to low activity levels is improved, and spatial resolution is maintained. A proposed instrument could be used for measuring other isotopes as well as fluorescent and visible stains.*

Investigators in many biological research laboratories are examining normal and abnormal human and animal proteins with one- and two-dimensional separation techniques to produce protein maps of plasma, urine, and other materials. Projects are under way to compile a complete protein index of humans (*1*). In other laboratories recombinant DNA techniques are being used to study the basic properties of DNA and to manipulate DNA fragments so as to "engineer" organisms that can manufacture scarce substances such as insulin, growth hormone, and interferon (*2*). Still other researchers are seeking to distinguish tissues, particularly tumors, or to detect genetic diseases (*3*). Essential to much of this research and development are the sequence analysis of DNA fragments and the screening of clones for specific genes. Both techniques require the detection of radioactive nucleic acid or antibody by autoradiography (*4*).

In the projects and techniques mentioned above, autoradiography or autofluorography is used in which x-ray film is exposed for hours or days in order to visualize the distribution and the amounts of labeled macromolecules. The

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