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Oxytocin, Vasopressin, and Prolactin Responses Associated With Nipple Stimulation

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ABSTRACT: Nipple stimulation by pregnant women close to term results in oxytocin release, as reflected by increased levels of oxytocin in peripheral plasma. This appears to be a specific response, and it does not involve either vasopressin or prolactin release.

HYPOTHALAMIC/PITUITARY responses to breast stimulation are different in the postpartum than in the antepartum period. In postpartum women, oxytocin and prolactin are integral parts of the milk ejection response.[1,2] Breast stimulation during suckling is known to result in the release of oxytocin. Similarly, prolactin is released in an episodic fashion during postpartum nipple stimulation. In contrast, these responses appear to be different in the antepartum period close to 40 weeks' gestation. In their recent study, Leake et al[3] could demonstrate no consistently positive oxytocin response during continuous nipple stimulation in the third trimester of pregnancy. On the other hand, results of other studies suggest that nipple stimulation causes a modest increase in maternal oxytocin concentrations.[4] With respect to prolactin, maternal concentrations of this hormone are elevated during pregnancy because of increased synthesis and release of this hormone. However, nipple stimulation in the antepartum period does not appear to result in an increase in maternal prolactin concentrations.[4]

Finally, it has not been determined whether vasopressin, another posterior pituitary hormone structurally similar to oxytocin that can bind to receptors in human myometrium,[5] is influenced by nipple stimulation in the antepartum period. Functionally, oxytocin[6] and possibly vasopressin[7] and prolactin[8] may be involved in smooth muscle stimulation. Moreover, antidiuresis has been reported to be associated with suckling in some animal species.[9,10] In human beings, however, vasopressin concentrations in the postpartum period are not increased with breast-feeding.[11] It is not known whether nipple stimulation in the antepartum period results in measurable changes in vasopressin concentrations.

Our study was designed to determine whether limited nipple stimulation by pregnant women at term results in a change in oxytocin, vasopressin, and prolactin concentrations in maternal plasma.

MATERIALS AND METHODS

Five patients at 37 to 42 weeks' gestation were included in this study. These patients were undergoing semi-weekly nonstress testing to assess fetal well-being.[12] Three patients were being evaluated for postmaturity, one patient for possible intrauterine fetal growth retardation, and one for history of hypertension. None of the patients were taking any drugs or medications. Because their nonstress tests were nonreactive, a contraction stress test by nipple stimulation was conducted to further evaluate the uteroplacental reserve.[13,14] All patients signed an informed consent form. The study had been approved by the Clinical Research Practices Committee at the Bowman Gray School of Medicine, Wake Forest University.

The contraction stress test by nipple stimulation was conducted essentially as described by Huddleston et al.[14] The patient was placed in the left lateral decubitus position, and fetal heart rate and uterine activity were monitored for approximately ten to 15 minutes by means of an electronic external fetal monitor (Hewlett Packard). At zero time, the patient began unilateral tactile stimulation of the nipple, which she continued for two minutes. In all cases, one or more contractions followed the nipple stimulation within seven minutes. A successful, normal nipple stimulation contraction stress test was accomplished in all of the patients, and pregnancy outcome was good.

Samples of venous blood were obtained in prechilled, heparinized syringes for measurement

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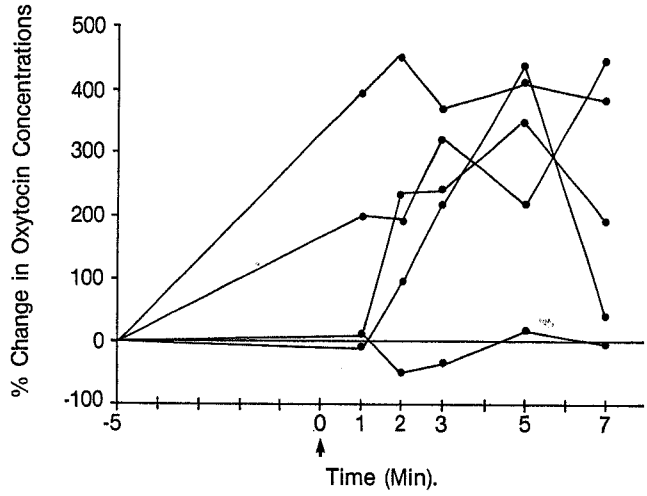

Percent change over baseline of oxytocin concentrations. Each curve represents individual patients sampled at indicated times. Onset of nipple stimulation is marked by arrow.

of oxytocin, vasopressin, and prolactin at five minutes before the onset of nipple stimulation and at 1, 2, 3, 5, and 7 minutes after the initiation of nipple stimulation. The blood samples were immediately centrifuged at 4 C and plasma was aspirated and stored at −30 C until assayed for the hormones. This was accomplished within six months in all cases.

Samples were assayed for oxytocin by a specific radioimmunoassay as previously described.[15]

Vasopressin was measured by a specific radioimmunoassay using methods previously reported.[16,17]

Prolactin was measured by an immunoradiometric assay (IRMA) system using two high-affinity monoclonal antibodies to prolactin (Prolactin, MAIAclone Serono Diagnostics). Samples were incubated with the first antiprolactin antibody for 15 minutes at 37 C. The second antibody was then thoroughly mixed in each tube. The mixture was incubated without any further mixing for five minutes at room temperature and the supernatant was separated using the MAIA magnetic separator provided by Serono. The supernatant was decanted from all tubes and the sediment was washed once with 0.5 ml of wash buffer. The supernatant was then decanted as before, and the sediment was counted in a gamma counter with an efficiency of 80% for ^{125}I. The intra-assay coefficient of variation was 5%, and the nonspecific binding was $<1.5\%$.

Statistical analysis of the maternal plasma concentrations of each one of these hormones was carried out by analysis of variance with repeated measures test. A difference from the baseline values was considered statistically significant if the P value was $<.05$.

TABLE. Maternal Concentrations* of Oxytocin, Vasopressin, and Prolactin

Time (min)	*Oxytocin (pg/ml)*	*Vasopressin (pg/ml)*	*Prolactin (ng/ml)*
−5	1.45 ± 0.68†,**,§	6.80 ± 0.54	159.52 ± 43.04
0	Nipple Self-Stimulation (0-2 min)		
+1	2.04 ± 0.67†	7.35 ± 1.65	153.37 ± 39.66
+2	2.35 ± 0.52	8.20 ± 1.93	154.24 ± 44.47
+3	2.84 ± 0.50§	8.40 ± 1.45	171.45 ± 40.83
+5	3.75 ± 0.80†,‡	7.25 ± 0.85	165.86 ± 37.71
+7	2.88 ± 0.67**	8.80 ± 1.15	169.90 ± 36.59

*Mean ± SEM.
†P = .033 by ANOVA and posthoc t test, showing a significant effect of time between t = −5 and t = +5 and t = +7.
**P = .05 by ANOVA and posthoc t test, showing a significant effect of time between t = −5 and t = +5 and t = +7.
‡P = .02 for t = +5 greater than t = +1 by ANOVA and posthoc t test.
§P = .581 between t = −5 and t = +3 by ANOVA and posthoc t test.

RESULTS

Nipple stimulation resulted in an increase in the plasma levels of oxytocin (Figure and Table). Moreover, in three of the five cases the rise was greater than 3 pg/ml and in four out of five, greater than 1 pg/ml. After the cessation of nipple stimulation, oxytocin concentrations fluctuated above the baseline. In two cases, they returned close to the baseline. The onset of uterine contractions, as noted on the external electronic fetal monitor, did not always coincide with the peak in oxytocin levels, but the onset did occur either with or after the maximal increase had been achieved.

In contrast to the results with oxytocin, vasopressin was not altered by nipple stimulation (Table). Finally, as expected for this stage of pregnancy, prolactin levels were uniformly elevated, but were not changed by nipple stimulation (Table).

DISCUSSION

This series of experiments describes changes in maternal concentrations of three pituitary hormones as a result of nipple stimulation in pregnant women at term. There are several reasons why they were measured simultaneously in the same subjects. First, nipple stimulation appears to result in differential stimulation of the hypothalamic/pituitary axis, depending on the maternal hormonal milieu (ie, antepartum vs postpartum). Second, both oxytocin and vasopressin are released by the posterior pituitary in response to different afferent stimuli. Oxytocin is released because of nipple stimulation during suckling, whereas vasopressin release is due to dehydration and increased plasma osmolarity. Nevertheless, both hormones can bind to membrane receptors in both the human uterus[5] and the mammary gland,[7] and may be physiologically important in the regulation of both myometrial contractility and milk ejection responses. Moreover, in some

species, suckling has been associated with antidiuresis, implicating a functional role for either oxytocin or vasopressin or both.[9,10] Third, prolactin is released by the anterior pituitary during lactation as a response to nipple stimulation. Prolactin might also have a stimulatory effect on myometrial contractility.[8] Thus, from several standpoints (myometrial contractility, milk ejection neuroendocrine reflex fluid balance), it was important to measure the effects of short-term nipple stimulation on the concentrations of these hormones during the antepartum period. From the clinical standpoint, since oxytocin and possibly the other two hormones may increase uterine contractility, a simultaneous release of all three might have resulted in uterine tetany, a potentially dangerous situation for fetal and maternal well-being. Nipple stimulation is carried out by many women in preparation for labor and delivery[13] and is used clinically as an indicator of fetal well-being.[18]

In the antepartum period at term, within seven minutes of the onset of maternal nipple stimulation, a differential response to oxytocin, vasopressin, and prolactin release is observed. During this interval, the only hormone released as a response to nipple stimulation is oxytocin. Neither vasopressin nor prolactin was shown to be regulated in this fashion.

With regard to oxytocin release, these observations are in agreement with those of some reports[4] but not others.[3] The causes of these discrepancies are not clear. In our study, the blood sampling intervals were shorter, and different oxytocin antiserum was used. However, even in those reports in which an inconsistent oxytocin response to nipple stimulation was observed, there were a few patients who did demonstrate positive responses. Furthermore, work by others has shown that there is a consistent, albeit modest, oxytocin response to nipple stimulation.[4] Whether this increase in peripheral levels of oxytocin might be responsible for increased uterine contractility, as suggested by our results, is not known.[19] End-organ responsiveness to oxytocin might be equally important. In this context, it has been shown that the number of myometrial oxytocin receptors increases dramatically shortly before the onset of term labor,[6] and that levels of oxytocin might not be correlated with the onset of uterine contractions.[20]

Although vasopressin-specific receptors are present in mammary gland and uterus, their function is not known. From our data, it does not appear that nipple stimulation results in an increase in vasopressin concentrations in the short term (seven minutes). A long-term effect appears unlikely, but it cannot be ruled out. This observation underscores the selectivity of the nipple stimulation-hypothalamic-pituitary neuroendocrine reflex. Finally, a possible relationship between vasopressin release and nipple stimulation causing antidiuresis is not present. Interestingly, some stimuli causing vasopressin release specifically (hemorrhage, hypertonic saline) have been reported to result in oxytocin release in conscious dogs.[21]

The absence of a stimulatory response in prolactin concentrations after nipple stimulation in the antepartum period is in agreement with previous observations.[4] This process, however, is reversed in the postpartum period, when, after the delivery of the placenta and the dramatic decrease in the levels of sex steroid hormones, nipple stimulation results in episodic prolactin release that, in the early postpartum period, reaches a maximum at 15 minutes after the start of nipple stimulation.[1] We doubt that extending our sampling period would have yielded an increase in prolactin concentrations, since the elevated prolactin levels we report are already in the range previously described.[1,4] Because our sampling did not extend beyond seven minutes, however, our conclusions must be limited to the period immediately after nipple stimulation.

Our observations suggest that nipple stimulation in pregnant women close to term results in oxytocin release, as reflected by increased levels of oxytocin in peripheral plasma. Given the short-term sampling period, this appears to be a specific response, and it does not involve either vasopressin or prolactin release. Since pregnancy outcome in these patients was relatively normal, these results may be applicable to pregnant women at term. Nevertheless, the fact that our patients were being evaluated for pregnancy-related problems should be kept in mind.

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