Serotonin Transport and Metabolism in the Mammary Gland Modulates Secretory Activation and Involution


Department of Molecular and Cellular Physiology (A.M.M., L.L.H., N.D.H.), and Systems Biology and Physiology Program (A.M.M., K.A.G., N.D.H.), University of Cincinnati, Cincinnati, Ohio 45267-0576; Division of Neonatology (L.A.N.-R.), Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio 45229; Department of Nutrition (K.G.D.), University of California at Davis, Davis, California 95616-8669; Department of Pediatrics (C.J.C.), University of California Davis Medical Center, Sacramento, California 95817; and James L. Winkle College of Pharmacy (K.A.G.), University of Cincinnati, Cincinnati, Ohio 45267

Context: Serotonin [5-hydroxytryptamine (5-HT)] is an important local regulator of lactation homeostasis; however, the roles for the serotonin reuptake transporter and monoamine oxidase have not been known.

Objective: The aim of the study was to determine whether drugs that impact 5-HT affect human lactation physiology.

Design and Setting: We conducted laboratory studies of human and animal models and an observational study of the onset of copious milk secretion in postpartum women at a university medical center.

Participants: We studied women expecting their first live-born infant; exclusion criteria were: referred to the medical center for another medical condition, known contraindication to breastfeeding, and less than 19 yr of age and unable to obtain parental consent.

Intervention(s): The mothers were interviewed. The cell and animal studies consisted of a variety of biochemical, pharmacological, and genetic interventions.

Main Outcome Measure(s): The human subjects outcome was prevalence of delayed onset of copious milk secretion. The cell and animal outcomes were physiological and morphological.

Results: Inhibiting serotonin reuptake in mammary epithelial cells altered barrier function, and the effects were amplified by coadministering a monoamine oxidase inhibitor. Direct delivery of fluoxetine by slow-release pellets caused localized involution. TPH1 knockout mice displayed precocious secretory activation. Among a cohort of 431 women, those taking SSRI were more likely (P = 0.02) to experience delayed secretory activation.

Conclusions: Medications that perturb serotonin balance dysregulate lactation, and the effects are consistent with those predicted by the physiological effects of intramammary 5-HT bioactivity. Mothers taking serotonergic drugs may need additional support to achieve their breastfeeding goals. (J Clin Endocrinol Metab 95: 837–846, 2010)
Breastfeeding benefits both infants and mothers in many ways (1–4). Despite these important health benefits, only 11% of mothers in the United States breastfeed exclusively for the recommended duration of 6 months (5). For mothers who initiate breastfeeding, the early postpartum period sets the stage for sustaining exclusive breastfeeding. Women who supplement with infant formula because of breastfeeding difficulties in the early postpartum period are likely to continue to supplement, and/or wean prematurely (6–9). One early breastfeeding difficulty that is particularly common among women in the United States is delayed secretory activation (a.k.a. delayed stage II lactogenesis) (10, 11). Secretory activation in women is defined as “the initiation of copious milk secretion” (12). Researchers commonly define delayed secretory activation in humans as occurring later than 72 h postpartum (7, 11). The biological mechanisms that contribute to delayed secretory activation remain poorly defined, although primiparous women are at a higher risk than multiparous women (11). Mothers who experience delayed secretory activation are at significantly greater risk for early cessation of breastfeeding (7, 13, 14). Therefore, if selective serotonin reuptake inhibitor (SSRI) use is found to be a significant risk factor for delayed secretory activation, it may be necessary for women taking an SSRI to receive additional guidance to meet their breastfeeding goals.

The initiating event for secretory activation is the functional assembly of high resistance tight junctions (TJ). Conversely, TJ disruption is required for glandular involution (15). Thus, mammary epithelial TJ are dynamically regulated not only during the secretory activation phase of lactation, but also during involution. Involution occurs via three sets of processes: 1) milk stasis-induced cessation of secretion combined with TJ disruption; 2) collapse of alveoli, with apoptosis of milk secreting cells; and 3) remodeling of the epithelium and adipose tissue (16). Of the plethora of molecules identified as regulators of involution (17), most are involved in apoptosis and tissue remodeling. The factors responsible for the autocrine/paracrine negative feedback on milk secretion and barrier function have remained elusive. Recently, our lab identified the monoamine serotonin [5-hydroxytryptamine (5-HT)] as being one of these molecules (18–20).

Serotonin action is controlled in two ways. Intracellularly, the amount of 5-HT is controlled by a balance between synthesis (tryptophan hydroxylase activity) and degradation [monoamine oxidase (MAO)]. Extracellularly, the availability of 5-HT is controlled by a recycling mechanism facilitated by the 5-HT transporter (SERT). The 5-HT transporter is the target of pharmaceuticals, particularly SSRIs. This class of drugs is commonly used to treat depression, including postpartum. We recently demonstrated that SERT is expressed in primary human mammary epithelial cells and an immortalized mammary epithelial cell line (MCF10A) (19, 21).

Milk stasis in the mammary glands induces 5-HT synthesis in the epithelium, where it affects TJ opening downstream of the 5-HT7 receptor (18, 19, 22). The actions of serotonin on TJ were shown to be biphasic (19, 20). A transient phase of TJ closure was followed by TJ opening after several hours. Although these previous studies established the effects and mechanisms of 5-HT on TJ, the physiological implications of altering 5-HT bioactivity within the tissue have largely been inferred from indirect evidence. Here, we show that drugs that enhance 5-HT activity locally, including SERT and MAO inhibitors, alter mammary epithelial functions. We also show that these effects are similar in human, mouse, and bovine systems. In the mouse, enhancement of 5-HT activity by inhibition of SERT caused precocious involution, whereas mice defective for 5-HT synthesis in the mammary gland [tryptophan hydroxylase 1 (TPH1) knockout] displayed hypersecretion and ductal ectasia. In addition, we present epidemiological evidence that women taking SSRI medications are at risk for delayed secretory activation.

**Subjects and Methods**

**Animal experiments**

All wild-type mice were of the C57BL/6J strain and were between 10 and 16 wk of age. Animals were housed in a barrier facility, and all procedures were approved by the Institutional Animal Care and Use Committee. Pregnancy d 1 is defined by observation of a vaginal plug. Lactation d 0 was the day of parturition, and involution d 1 was 24 h after forced weaning. Bovine mammary cell preparations were a generous gift from Robert J. Collier at the University of Arizona. Primary human mammary epithelium obtained from reduction mammoplasty under Institutional Review Board approval was a generous gift from Eric R. Hugo at the University of Cincinnati.

Ethylene vinyl acetate resin (Elvax; DuPont, Wilmington, DE) pellets were synthesized according to previously published protocols (23). All pellets contained 24 μg fluoxetine (FLX) per milligram of pellet weight. Anesthetized mice were implanted with a 1.0- to 1.5-mg pellet at d 8–9 postpartum. Pellets containing FLX were implanted in the no. 3 and 4 glands, whereas the contralateral glands received vehicle control pellets. India ink marked the implantation site. Proximal (≤0.5 mm) and distal (≥5 mm) were defined relative to the pellet implantation site (i.e., India ink). After recovery, mothers were returned to their litters (normalized to seven to eight pups) and resumed normal nursing habits as evidenced by the pup’s milk-filled stomachs. ImageJ software was used for lumen diameter measurements (six alveoli from each section (three per mammary gland) for each time point (three mammary glands per time point).

TPH−/− mice and the corresponding controls (TPH+/+) were congenic (C57Bl/6J) and were each grafted with a wild-type...
anterior pituitary gland under the renal capsule at 8 wk of age. Grafts remained for 18 d, after which the mice were killed and mammary glands removed.

**Cell culture**

Primary human mammary epithelial cells were cultured using previously described methods (24). MCF10A cells on Transwells (Corning Inc., Corning, NY) were cultured as previously described (21). Primary human mammary epithelial cell Transwells were similarly cultured, but on collagen-coated polyester membranes at 2.5 × 10^5 cells/cm². Tissue dissociation and isolation of bovine epithelial cells was performed as previously described (25). Primary bovine mammary epithelial cells were maintained on collagen-coated plastic, then transferred onto polyester Transwells.

**Immunocyto(-histo)chemistry and antibodies**

Transwell cultures were fixed by 4% paraformaldehyde. Sections were incubated in borate buffer (pH 8.5) (80 mM boric acid, 20 mM sodium borate) overnight at 75 °C for antigen retrieval followed by antibody incubations. Mammary tissues were fixed in 4% paraformaldehyde and sectioned on cryostat. Sections were rehydrated, blocked with goat antimouse F(ab) unlabeled antibody and 10% normal serum, followed by antibody incubations. Antibodies used were monoclonal anti-SERT antibody (Advanced Targeting Systems, Inc., San Diego, CA), antioccludin (Zymed Laboratories, South San Francisco, CA), antimouse IgG Alexa Fluor 488 (Molecular Probes, Eugene, OR), and antitrabibit IgG Alexa Fluor 546 (Molecular Probes). TOPRO-3 iodide (Molecular Probes) was used to stain nuclei.

**Epidemiology data collection and analysis**

All expectant primiparous women receiving prenatal care at a University of California, Davis Medical Center clinic between January 2006 and December 2007 were screened for study eligibility. Selection criteria were: expecting first live-born infant, between 32 and 40 wk gestation at time of interview (mean = 35.9 wk, sd = 1.6 wk), single fetus, speaks either English or Spanish, and ZIP code within 8-mile radius of the Medical Center (Sacramento, CA). Exclusion criteria were: referred to the Medical Center due to medical condition, known absolute contraindication to breastfeeding, or younger than 19 yr of age and not able to obtain parental consent. The study protocol and consent form were approved by the University of California Davis Institutional Review Board. Consenting subjects were interviewed at a prenatal clinic visit regarding demographic information; health history, including current medication use; depressive symptoms; infant feeding knowledge and attitudes; and infant feeding intentions (26). Participants were recontacted in the hospital between 72 and 96 h postpartum (“day 3”) to assess breastfeeding experience, including infant breastfeeding behavior (27) and the timing of the secretory activation. The latter, which is characterized by the onset of copious milk production, was assessed as described previously (11), which is based on the maternal report of when her breasts felt “noticeably fuller” on a 1 to 5 scale, (where 1 = no change since giving birth, 3 = noticeably fuller, and 5 = uncomfortably full). For participants who had not experienced “noticeable fullness” by the time of the day 3 interview, the question was repeated at the day 7 interview. Delayed secretory activation was defined as maternal perception of onset of noticeable fullness beyond 72 h postpartum. This approach has been previously validated (7). The characteristics of women in the SSRI vs. non-SSRI groups were compared using Fisher’s exact test for dichotomous variables and Student’s t test for continuous variables. The survival distribution functions for timing of onset of secretory activation were compared between groups using Kaplan-Meier estimator methods (SAS Proc Lifetest; SAS Institute, Cary, NC). The proportion in each group with delayed secretory activation was first compared using Fisher’s exact test. Further examination after adjustment for potentially confounding baseline characteristics was done using the Mantel-Haenszel statistic (a weighted average analysis).

**Results**

**Contribution of SERT and MAO to intrinsic 5-HT activity in MCF10A cells**

Previous in vitro studies using exogenous 5-HT and TPH1 knockout mouse cells implied that mammary gland 5-HT could regulate lactation function, but there was no direct proof that 5-HT secreted endogenously is sufficient to regulate epithelial functions. To confirm that endogenous 5-HT regulated epithelial TJ, we tested the consequence of blocking 5-HT reuptake in MCF10A cells with FLX, a well-characterized SSRI. We confirmed that SERT was present in MCF10A cell membranes with confocal microscopy, and we show here that SERT is highly restricted to the apical membrane of the superficial cell layer and is bounded by occludin (a TJ protein) staining (Fig. 1A).

Transepithelial electrical resistance (TEER), which is inversely related to the permeability of TJ, was monitored daily. FLX elicited a biphasic response of TEER (Fig. 1B). At low concentrations and/or earlier time points, FLX caused a potentiation of TEER, whereas at later times and/or higher concentrations of FLX the TEER was decreased. Specifically, at a concentration of 10 μM FLX, TEER was increased approximately 45% and remained elevated for 3 d (Fig. 1B). At 30 μM FLX, TEER increased on d 1 and subsequently declined on d 2–3. At a higher concentration (100 μM), TEER declined by 24 h. Overall cell viability was not affected at any FLX dose. This biphasic pattern in TEER was similar to that observed in MCF10A Transwell cultures treated with varying times and concentrations of exogenous 5-HT (20).

MAO enzymatically inactivates 5-HT, and thus could affect the TEER changes observed with FLX. We therefore tested the consequences of treating cells with the MAO inhibitor phenelzine (PHLZ) alone or in combination with FLX. FLX was used at a concentration of 10 μM, which resulted in increased TEER at 48 h (Fig. 1C). At this time point, PHLZ alone caused a small, nonsignificant increase
in TEER ($P \geq 0.05$). In combination with FLX, there was a highly significant decrease in TEER, indicating that the combination of drugs increased serotonin activity to levels in the TEER-inhibitory range ($P < 0.01$; Fig. 1C).

Dexfenfluramine (FEN) causes the release of monoamines from stored vesicles to increase the extracellular concentration of 5-HT. When administered to MCF10A cells, FEN caused a decrease in TEER similar to that seen with the combination of PHLZ and FLX (Fig. 1C). The responses to serotonergic drugs (FLX, PHLZ, FEN) in primary human and primary bovine Transwell membranes was comparable to that in MCF10A (supplemental Figure S1, published as supplemental data on The Endocrine Society’s Journals Online website at http://jcem.endojournals).

To confirm that a decrease in TEER represented TJ disruption, we immunostained for the TJ scaffolding protein ZO-1 in FLX-treated (30 $\mu M$) cultures in which the resistance was decreased by approximately 60% (d 3). In a high-resistance barrier culture, ZO-1 staining distinctly circumscribed the cells and was predominantly membrane-associated (Fig. 1Di). After FLX treatment and corresponding to decline in TEER, ZO-1 staining became intracellular, and observable gaps in the circumscribing ZO-1 immunostaining could be seen (Fig. 1Dii).

Expression and localization of SERT in vivo

Having shown that 5-HT turnover regulates the epithelial barrier in human and bovine mammary epithelial cells through in vitro studies, we were interested in demonstrating the effects of SERT antagonism in the mammary gland in vivo. As a basis for these studies, we wanted to document SERT protein in the rodent mammary gland. Serotonin transporter protein was present in mammary gland extracts at all stages of postpubertal development (Fig. 2A). SERT levels were increased by d 1 of lactation and remained elevated throughout lactation (Fig. 2B).

In vivo, the mouse mammary gland epithelium (d 10 postpartum) expressed SERT on the apical aspect of the epithelial cells (Fig. 2C, arrow), consistent with the distribution in mammary epithelial cells in Transwell cultures. Additional SERT immunostaining was observed in the mammary stroma (both in vascular and nonvascular elements; data not shown). Unlike epithelial SERT, for which we have in vitro functional data, the functions of extraepithelial SERT have not yet been studied.

In vivo blockade of 5-HT reuptake during lactation

Having established that FLX can affect TJ and that SERT is expressed in the mammary glands, we sought to establish the consequences of directly treating the mammary gland with FLX. Implantable pellets were used so that FLX could be delivered to the mammary gland without systemic effects, and each gland could serve as an internal control (24, 28). We constructed pellets containing FLX (24 $\mu g$/mg dry pellet weight), which were implanted...
into mammary glands of lactating mice. After surgery, the mothers were returned to their pups and resumed normal nursing habits.

Over the time course studied, the mammary gland tissue surrounding the control pellets was histologically indistinguishable from normal lactating tissue. These tissues were characterized by distended alveoli filled with milk, surrounded by basophilic epithelial cells (Fig. 3A, “Vehicle Proximal”). Specimens from areas of the drug-treated glands that were as far as reasonable from the marked pellet sites were also analyzed (Fig. 3A, “Fluoxetine Distal”). These areas were very similar in histological appearance to the control-implanted glands. In contrast, glandular morphology was starkly altered in the tissue surrounding the FLX pellet (Fig. 3A, “Fluoxetine Proximal”). Moreover, these alterations were time dependent. On the first day after implantation, alveoli proximal to the FLX pellet began to collapse, and they continued to do so through d 3. By d 4, the beginning of adipose tissue redifferentiation and extensive tissue remodeling became apparent in these regions (Fig. 3A, “Fluoxetine Proximal”). Importantly, these changes occurred in the context of continued positive endocrine influences brought on by suckling. Figure 3B shows quantification of the alveolar lumen diameters in the three treatment groups through d 3. Alveoli distal to the FLX pellet tended to be somewhat smaller than those in the control glands, but the difference was not statistically significant and did not change over time. In contrast, luminal diameters of alveoli proximal to FLX pellets were significantly smaller and trended downward over the 3-d period (Fig. 3B). Because alveoli could not be defined structurally in the FLX-treated glands on d 4, there was no quantification of these specimens.

Secretory status of TPH1 knockout mammary glands

The foregoing in vivo study showed that enhancing 5-HT bioavailability (FLX) caused involution-like changes. The corollary hypothesis, suggested also by previously published in vitro data (18), was that reduced 5-HT bioavailability would favor secretory activation. We tested this hypothesis by causing hyperprolactinemia (pituitary grafting) in mice that were either wild-type (TPH1+/+) or 5-HT deficient (TPH1−/−). In our experience, pituitary grafting (for review, see Ref. 29) for 18 d causes pregnancy-like alveolar development, without evidence of secretory activation. Correspondingly, TPH1+/+ mice with pituitary grafts had well-developed glands with extensive alveolar development (Fig. 4A) but narrow ducts without large volumes of secretory material (Fig. 4, B and C). In contrast, TPH1−/− mice had similarly extensive alveolar development (Fig. 4D) but displayed distended ducts and alveoli filled with secretory material (Fig. 4D, arrow and arrowhead, respectively). The luminal fluid was grossly indistinguishable from milk and contained numerous lipid globules (Fig. 4, E and F). The development of the mammary gland in pituitary-grafted TPH1−/− mice was consistent with the hypothesis of precocious secretory activation.

Assessment of SSRI as a risk factor for delayed secretory activation

Based on our findings in human cell cultures and animal models, we hypothesized that women taking SSRI medications (elevated net 5-HT bioavailability) might be at a greater risk for delayed secretory activation, which requires TJ closure and definitive milk biosynthesis (30, 31). Data were collected as part of a longitudinal cohort study examining barriers to early lactation success. The study group was a multiethnic population of primiparous (i.e. first live birth) women (32, 33).

Over the 24 months of study enrollment, 768 of those screened met the eligibility criteria, and 532 of these women agreed to the prenatal interview (69% of those eligible). Of the 532 women enrolled prenatally, 40 (7.5%) were lost to follow-up by the time of delivery, and 44 became ineligible for continued follow-up (preterm birth, n = 11; unable to initiate feeding at the breast within 24 h for medical reasons, n = 21; and mother chose not to initiate breastfeeding, n = 12). Thus, 448 breastfeeding mother-infant pairs remained in the study for postnatal follow-up.
In the study group, 431 patients (96%) yielded data on the timing of secretory activation, including eight women (1.9%) who indicated regular use of an SSRI medication (FLX, n / 11005; paroxetine, sertraline, citalopram, escitalopram, and duloxetine, n / 11005 for each). The characteristics of the SSRI and non-SSRI mothers are shown in Table 1. Women were similar in most characteristics, except that women in the SSRI group were significantly more likely to have scored in the “at-risk” range on the Center for Epidemiologic Study depressive symptoms scale (CES-D) (34) (as expected) and were somewhat (but not significantly) more likely to be obese or to have delivered by cesarean section. Figure 5 shows the timing of secretory activation in the SSRI group vs. the non-SSRI group. Median onset of secretory activation was 85.8 h postpartum in the SSRI-treated mothers, vs. 69.1 h in the non-SSRI mothers (P = 0.004). All women in the SSRI group had experienced secretory activation by the d 7 interview. Among women in the non-SSRI group, 2.1% at d 3 and 1.7% at d 7 were censored. Delayed secretory activation occurred in seven of eight women in the SSRI group (87.5%), compared with 43.5% of women in the non-SSRI group [relative risk (95% confidence interval), 2.0 (1.51–2.67); Fisher’s exact test, two-tailed P value / 11021 = 0.02]. As shown in Table 2, the relative risk of delayed secretory activation remained significantly higher (for all, P < 0.05) in the SSRI group after Mantel-Haenszel adjustment for potentially confounding factors, including maternal age, maternal obesity, cesarean section delivery, and infant gestational age, or infant breastfeeding behavior (for all, P < 0.04). There was a single subject in the SSRI group who did not meet the a priori definition of delayed secretory activation (>72 h postpartum), and her secretory activation occurred at 72.0 h.

Discussion
Previous studies on the actions of 5-HT on the mammary glands or mammary epithelial cultures have primarily used exogenous agonists and/or antagonists (18–20, 22).

FIG. 3. Effects of Elvax FLX pellet implants into lactating mammary glands. A, Representative hematoxylin and eosin-stained sections of mammary glands after Elvax pellet implantation: d 1 through d 4 of vehicle- (left column) and FLX- (center and right columns) treated lactating mouse mammary glands. Proximal (≥0.5 mm) and distal (≥5 mm) are relative to the pellet implantation site. Scale bar, 75 μM. B, Mean (±SEM) lumen diameters (n = 6 per section per mammary gland) from vehicle- and FLX-treated lactating mouse mammary gland pellet implants (n = 3 mice × 2 mammary glands/mouse/day). Data were analyzed by one-way ANOVA followed by Dunnett’s test to compare each group with the vehicle control for that day (*, P < 0.01).
Experiments that relied on exogenous receptor-active agents (agonists or antagonists) could not answer directly whether the autocrine secretory turnover of 5-HT was sufficient to explain feedback inhibition in the mammary gland epithelium. We have manipulated the intrinsic 5-HT bioactivity of the mammary gland using agents that were predicted to alter the rates of turnover of the endogenous ligand. These included drugs that inhibit SERT or MAO (FLX and PHLZ, respectively), or that activate vesicular release (FEN). Therefore, the current in vitro results demonstrate the importance of the dynamic turnover of 5-HT content within the mammary gland tissue. In vivo results confirm that altering 5-HT bioavailability affects the function of the glands in the whole organism.

Transwell cultures provide a reliable platform for studying polarized mammary epithelial cells (21). Here we cultured MCF10A, primary human, and primary bovine mammary epithelial cells on Transwells and demonstrated consistent TJ responses to serotonergic drugs. TJ assembly is the initiating event for secretory activation. During lactation, TJ are dynamically regulated in accordance with the degree of milk stasis, where prolonged stasis leads to involution. Using Transwell cultures, serotonin (19, 20), and now serotonergic drugs, have been shown to biphasically modulate TJ integrity.

The 5-HT transporter, SERT, is the target for the most commonly prescribed class of antidepressants in the United States and other developed countries (35). Here we have demonstrated that SERT is expressed in mammary tissue and specifically that SERT is present in the apical membranes of mammary epithelial cells. Pharmacological antagonism of SERT in vivo affected the morphology of mammary tissue, leading to a local, involution-like state. Before the current study, reports on SSRI drug use during pregnancy and lactation focused on whether SSRI caused developmental defects in utero (36–38) or was passed into the milk during lactation (39, 40) and on the safety im-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-SSRI group</th>
<th>SSRI group</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>26.1 (6.0)</td>
<td>28.8 (6.6)</td>
<td>0.28</td>
</tr>
<tr>
<td>≥30 yr old</td>
<td>27.2%</td>
<td>37.5%</td>
<td>0.69</td>
</tr>
<tr>
<td>Attended college</td>
<td>59.8%</td>
<td>62.5%</td>
<td>1.00</td>
</tr>
<tr>
<td>Public health insurance</td>
<td>48.9%</td>
<td>50.0%</td>
<td>1.00</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.7 (6.1)</td>
<td>31.9 (8.4)</td>
<td>0.17</td>
</tr>
<tr>
<td>BMI in obese range (≥30.0 kg/m²)</td>
<td>31.7%</td>
<td>57.1%</td>
<td>0.22</td>
</tr>
<tr>
<td>Depressive symptoms score ≥16°</td>
<td>17.5%</td>
<td>50.0%</td>
<td>0.04</td>
</tr>
<tr>
<td>Cesarean section delivery</td>
<td>30.5%</td>
<td>62.5%</td>
<td>0.11</td>
</tr>
<tr>
<td>Breastfed ≥8 times in first 24 h</td>
<td>85.5%</td>
<td>100%</td>
<td>1.00</td>
</tr>
<tr>
<td>Infant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>39.6 (1.0)</td>
<td>38.9 (1.1)</td>
<td>0.05</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3.38 (0.43)</td>
<td>3.41 (0.40)</td>
<td>0.86</td>
</tr>
<tr>
<td>Apgar score, 1 min</td>
<td>7.7 (1.5)</td>
<td>7.8 (0.9)</td>
<td>0.95</td>
</tr>
<tr>
<td>Apgar score, 5 min</td>
<td>8.8 (0.5)</td>
<td>8.6 (0.5)</td>
<td>0.27</td>
</tr>
<tr>
<td>&gt;60 ml formula, 0–48 h postpartum</td>
<td>30.5%</td>
<td>37.5%</td>
<td>0.69</td>
</tr>
<tr>
<td>Suboptimal breastfeeding behavior†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>62.3%</td>
<td>62.5%</td>
<td>1.00</td>
</tr>
<tr>
<td>Day 3</td>
<td>28.2%</td>
<td>62.5%</td>
<td>0.05</td>
</tr>
<tr>
<td>Day 7</td>
<td>22.8%</td>
<td>25.0%</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD) or percentage. BMI, Body mass index.

* Student’s t test for comparison of means; Fisher’s exact test for comparison of proportions.

† Cutoff for at risk of depression based on CES-D scale (Center for Epidemiologic Study).

‡ According to Infant Breastfeeding Assessment Tool score ≥10 (27).

![FIG. 5. Timing of onset of stage II lactogenesis](https://academic.oup.com/jcem/article-abstract/95/2/837/2597380/502222)
TABLE 2. Delayed secretory activation by SSRI group, stratified by select baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-SSRI group</th>
<th>SSRI group</th>
<th>M-H adjusted RR [95% CI]a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>184/23 (44%)</td>
<td>7/8 (88%)</td>
<td>2.01 [1.51–2.67]</td>
</tr>
<tr>
<td>Maternal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not obese (BMI &lt;30 kg/m²)</td>
<td>104/72 (38%)</td>
<td>3/3 (100%)</td>
<td>1.84 [1.31–2.58]</td>
</tr>
<tr>
<td>Obese (BMI ≥30 kg/m²)</td>
<td>67/126 (53%)</td>
<td>3/4 (75%)</td>
<td></td>
</tr>
<tr>
<td>Excessive depressive symptoms (score ≥16)c</td>
<td>151/349 (43%)</td>
<td>4/4 (100%)</td>
<td>2.00 [1.49–2.67]</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>33/74 (45%)</td>
<td>3/4 (75%)</td>
<td></td>
</tr>
<tr>
<td>Cesarean section delivery</td>
<td>117/294 (40%)</td>
<td>3/3 (100%)</td>
<td>1.85 [1.38–2.49]</td>
</tr>
<tr>
<td>Infant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age ≥40 wk</td>
<td>77/175 (44%)</td>
<td>7/7 (100%)</td>
<td>2.00 [1.49–2.71]</td>
</tr>
<tr>
<td>Gestational age &lt;40 wk</td>
<td>105/242 (43%)</td>
<td>0/1 (0%)</td>
<td></td>
</tr>
<tr>
<td>Optimal breastfeeding behavior, day 3</td>
<td>120/296 (41%)</td>
<td>3/3 (100%)</td>
<td>1.96 [1.46–2.63]</td>
</tr>
<tr>
<td>Suboptimal breastfeeding behavior, day 3d</td>
<td>55/116 (47%)</td>
<td>4/5 (80%)</td>
<td></td>
</tr>
</tbody>
</table>

Data for non-SSRI and SSRI groups are expressed as number delayed/total number (% delayed). BMI, Body mass index.

a Baseline characteristics where P value <0.25 for the comparison between SSRI groups.

b Mantel-Haenszel adjusted relative risk [95% confidence interval], comparing the prevalence of delayed secretory activation between SSRI groups, adjusted for differences in the baseline characteristic shown.

c Cutoff for at risk of depression based on CES-D scale (Center for Epidemiologic Study).

d According to Infant Breastfeeding Assessment Tool score ≥10 (27).

plications of SSRI in milk (41, 42). Our data are the first to report another important aspect of SSRI use during the peripartum. One limitation of the in vitro studies is the difficulty in relating in vitro concentrations of FLX with what would be encountered in vivo from antidepressant therapy. To address this, we performed in vivo studies in both mice and women.

The risk of delayed secretory activation was 2-fold greater among primiparous women using an SSRI medication, compared with women in the same cohort not using an SSRI medication. Seven of eight women in the SSRI group experienced delayed secretory activation, with the remaining woman in the SSRI group experiencing onset of secretory activation at 72.0 h, the defined cutoff for delayed secretory activation. Although the fraction of women taking an SSRI medication was small, the 2-fold greater risk of delayed secretory activation was significant and remained significant after adjustment for baseline differences between SSRI groups. Nevertheless, further examination of this relationship with larger numbers of women using SSRI medications during the peripartum would be important, as would studies that could compare effects of different antidepressant medicines.

Understanding the physiology of lactation, and especially the factors that inhibit lactation, is important in several contexts, particularly for human breastfeeding and dairy production. In addition to the obvious goal of enhancing milk production, it is equally important to shut down the gland quickly at the end of the lactation period. The successful completion of the “dry period” in dairy cows is crucial to maximize milk production in subsequent lactations (43). Currently, it takes about 3 d for the cow udder to cease leaking milk after milking is suspended (44). During this dry-off period, dairy animals are susceptible to mastitis (45). Similarly, women who stop breastfeeding for medical reasons are also at increased risk for mastitis (46, 47). Understanding the physiology surrounding dry-off may provide novel approaches for ameliorating the negative consequences during this fragile period of the lactation cycle in dairy animals and women.

In conclusion, we have now demonstrated the importance of the intrinsic mammary gland 5-HT system that balances secretory activation with involution. Drugs enhancing 5-HT action cause TJ disassembly in vitro and involution-like changes in vivo. 5-HT-deficient mammary glands display precocious secretory activation and fluid accumulation upon hormonal stimulation. Furthermore, primiparous women taking an SSRI medication were more likely to experience delayed secretory activation, possibly through local 5-HT-dependent mechanisms.

Acknowledgments

The authors thank Erin Pangallo, Vaibhav Pai, Eric Hugo, Melissa Orr, and Archie Vomachka for technical contributions. We also thank Robert Collier for providing the primary bovine cells.

Address all correspondence and requests for reprints to: Nelson D. Horsemans, 231 Albert Sabin Way, Molecular and Cellular Physiology, Cincinnati, Ohio 45267-0576. E-mail: nelson.horsemans@uc.edu.

This work was supported by grants from the National Institutes of Health (DK52134 to N.D.H. and DK54966 to K.A.G.), a predoctoral fellowship (HD007463 to A.M.M.), and a postdoctoral fellowship (CA059268 to L.L.H.). This project was also...
supported by National Research Initiative Competitive Grant 2007-35206-17898 from the U.S. Department of Agriculture Cooperative State Research, Education, and Extension Service. The collection of epidemiological data was supported by Grant R40 MC 04294 from the Maternal and Child Health Bureau (Title V, Social Security Act), Health Resources and Services Administration, Department of Health and Human Services.


References


