Regular Article

Serotonin Regulates β-Casein Expression *via* 5-HT$_7$ Receptors in Human Mammary Epithelial MCF-12A Cells

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We previously reported that serotonin (5-hydroxytryptamine; 5-HT) suppresses β-casein expression, a differentiation marker in mammary epithelial cells, *via* inhibition of the signal transducer and activator of transcription 5 (STAT5) phosphorylation in the human mammary epithelial cell line, MCF-12A. In this study, we investigated the expression pattern of the different 5-HT receptor subtypes in MCF-12A cells, and identified the receptors involved in 5-HT-mediated suppression of β-casein protein expression. β-Casein mRNA expression was inhibited by 30 μM 5-HT in a time-dependent manner. Treatment with 30 μM 5-HT for 72 h decreased β-casein protein levels and STAT5 phosphorylation (pSTAT5). The cells expressed four 5-HT receptors subtypes (5-HTR$_{1D, 2B, 3A, and 7}$) at the mRNA and protein level, and their expression was elevated by prolactin (PRL) treatment. Additionally, the mRNA levels of 5-HTR$_{1D}$ and 5-HTR$_7$ were significantly higher than the other 5-HT receptors in the cells. Tryptophan hydroxylase 1 mRNA was detectable in the cells in the absence of PRL, and PRL treatment significantly increased its expression. β-Casein and pSTAT5/STAT5 levels in the cells co-treated with 5-HT and a selective 5-HTR$_{1D}$ inhibitor, BRL15572, were equal to those observed in cells treated with 5-HT alone. However, in the cells co-treated with 5-HT and a selective 5-HTR$_7$ inhibitor, SB269970, β-casein and pSTAT5/STAT5 levels increased in a SB269970 concentration-dependent manner. In conclusion, we showed that 5-HT regulates β-casein expression *via* 5-HTR$_7$ in MCF-12A human mammary epithelial cells.

Key words serotonin; β-casein; 5-hydroxytryptamine (5-HT)$_7$ receptor; human mammary gland; milk protein

Milk production and secretion are regulated by dynamic interactions between numerous endocrine hormones, including prolactin (PRL) and estrogen, and locally produced factors, such as sucking, which can induce PRL secretion. Suckling-induced PRL promotes mammary epithelial cell division and differentiation, and increases the synthesis of milk constituents.[1] It is known that signaling *via* the Janus kinase 2/signal transducer and activator of transcription 5 (Jak2/STAT5) pathway is involved in milk production.[2–5] PRL induces the phosphorylation of STAT5 *via* Jak2 activation, which stimulates the expression of genes encoding milk proteins, such as β-casein and whey acidic protein, which are important differentiation markers in mammary epithelial cells.[2–5]

Serotonin (5-HT) is thought to be an autocrine/paracrine regulator of lactation in many species, including mice, cows, and humans. Intrinsic 5-HT produced by the mammary epithelium plays an important role in the control of milk production. When the mammary gland is filled with milk during lactation, 5-HT provides negative feedback signals that suppress further milk synthesis in the mammary epithelium.[6,7] Reduced demand for milk during weaning can stimulate this negative feedback, initiating mammary gland involution and reducing milk production.[8] Previous studies using mammary epithelial cell culture models showed that exogenous 5-HT treatment led to increased tight junction permeability *via* 5-HT receptor 7 (5-HTR$_7$), and that this alteration is an important event in the initiation of mammary involution.[9,10] Additionally, 5-HT signaling caused cell shedding and increased active caspase-3 in the human mammary epithelial cell line MCF-10A, suggesting that 5-HT can induce apoptosis in mammary epithelial cells at the beginning of involution.[11] In contrast, recent studies showed that 5-HT stimulated synthesis and enhanced secretion of parathyroid hormone-related peptide (PTHrP), which is a calcium-mobilizing hormone. PTHrP is an endocrine factor that is synthesized within the mammary gland during lactation, and is responsible for calcium mobilization from the bone to milk to maintain calcium supplies for the infant.[12–14]

In our previous study, PRL treatment induced β-casein expression in the human mammary epithelial cell line MCF-12A *via* activation of the Jak2/STAT5 pathway. We also showed that 5-HT treatment decreased β-casein levels, with a concomitant reduction in STAT5 phosphorylation (pSTAT5),[15] suggesting that suppression of β-casein by 5-HT was associated with reduced STAT5 phosphorylation. These results implied that endogenous 5-HT in MCF-12A cells may be secreted and signal in an autocrine and/or paracrine manner in MCF-12A cells.

5-HT can exert its action through a repertoire of over 15 different receptors, depending on the species, belonging to seven families (5-HTR$_{1–7}$).[16] mRNA encoding four 5-HTR isoforms (5-HTR$_{1D, 2B, 3A, and 7}$) are expressed in primary human mammary epithelial cells.[17] Additionally, mRNA expression of five 5-HTRs (5-HTR$_{1B, 2A, 2B, 4, 7}$) is detectable in primary bovine epithelial cells, and agonists of 5-HTR$_{1B}$,

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5-HTR_{2A}, and 5-HTR_7 stimulated the expression of β-casein mRNA.[18] However, the 5-HTR subtypes involved in suppression of β-casein expression in human mammary epithelial cells have not yet been identified.

In the present study, we investigated whether the 5-HTR regulates β-casein expression in the human mammary epithelial cell line MCF-12A. Our data indicate that 5-HTR_7 is involved in 5-HT-mediated suppression of β-casein protein expression.

MATERIALS AND METHODS

Cell Culture MCF-12A cells were cultured as previously described.[15] Briefly, the cells were seeded at a density of 1×10^5 cells/cm^2 on dishes coated with Matrigel® (BD Biosciences, MA, U.S.A.), and were cultured in growth medium (GM) consisting of DMEM : F12 (1 : 1, Invitrogen, CA, U.S.A.) supplemented with 10 μg/mL human insulin (Sigma-Aldrich, MO, U.S.A.), 0.5 μg/mL hydrocortisone (Sigma-Aldrich), 20 ng/mL human recombinant epithelial growth factor (hEGF; BD Biosciences), 5% horse serum (Invitrogen, CA, U.S.A.), 100 IU/mL penicillin, and 100 μg/mL streptomycin (Sigma-Aldrich). Unless otherwise stated, the GM was changed to differentiation medium (DM), modified by the addition of 0.1 μg/mL PRL and the removal of hEGF, 24 h after cell seeding for 7 d. For treatment with 5-HT and/or 5-HTR antagonists, no horse serum was added to the DM. 5-HT and/or the antagonists were added to the DM on day 7 in culture for 24 to 96 h.

Quantitative Real-Time (RT) Polymerase Chain Reaction (PCR) Total RNA was isolated from cultured cells, and cDNA was prepared as previously described.[15] The mRNA levels were evaluated using a 7500 RT-PCR System (Applied Biosystems, CA, U.S.A.) with TaqMan Universal Master Mix II (Applied Biosystems), under the following conditions: 2 min at 50°C, 10 min at 95°C, followed by 60 cycles of 95°C for 15 s and 60°C for 1 min. Commercially available (Applied Biosystems) pre-designed primer and probe sets were used for human β-casein (*CSN2*) (Hs_00914395_m1), *5-HTR*$_{1D}$ (Hs_00704742_s1), *5-HTR*$_{2B}$ (Hs_00168362_m1), *5-HTR*$_{3A}$ (Hs_00168375_m1), *5-HTR*$_7$ (Hs_04194798_s1), tryptophan hydroxylase 1 (*TPH1*) (Hs_00188220_m1), and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) (Hs_02758991_g1). Quantitative values were obtained from the threshold cycle number. The mRNA level of each target gene was normalized to the *GAPDH* mRNA content for each sample. All samples were analyzed in triplicate, and the average cycle threshold values were used for data analysis.

Western Blot Analysis Total protein was extracted from cultured cells using the M-PER® Mammalian Protein Extraction Reagent (Pierce, IL, U.S.A.), and protein concentrations were determined using a BCA™ Protein Assay Kit (Pierce). Proteins were subjected to electrophoresis on 7.5% or 12.5% e-PAGEL (ATTO, Tokyo, Japan), and then transferred onto polyvinylidene difluoride membranes (GE Healthcare, Tokyo, Japan). The membranes were probed overnight at 4°C with primary antibodies specific for β-casein (1 : 400; Novus Biologicals, CO, U.S.A.), STAT5 (1 : 1000; Abcam, Tokyo, Japan), pSTAT5 (1 : 200; Abcam), 5-HTR_{1D} (1 : 200; Abcam), 5-HTR_{2B} (1 : 500; Novus Biologicals), 5-HTR_{3A} (1 : 500, Abcam), 5-HTR_7 (1 : 100, Abcam), or GAPDH (1 : 2000; Calbiochem, CA, U.S.A.). The membranes were then incubated with horseradish-peroxidase-conjugated secondary antibodies (1 : 2000; Santa Cruz, TX, U.S.A.), and immunoreactive bands were visualized using an Image Reader LAS-3000 System (Fuji Photo Film, Tokyo, Japan). Band density was analyzed using Image J ver.1.47 software.

Statistical Analysis All reported values were expressed as the mean±standard error of the mean (S.E.M.). Student's *t*-test was used to compare two groups. For comparisons between multiple groups, data were analyzed by one-way ANOVA, followed by Dunnett's test or the Tukey–Kramer test to compare each group. Differences were considered statistically significant when $p<0.05$.

RESULTS

5-HT Treatment Decreases β-Casein Expression in MCF-12A Cells We previously showed that treatment with 100 μM 5-HT for 72 or 96 h decreased β-casein mRNA expression in MCF-12A cells.[15] Cells were grown for 7 d (in GM for 1 d and in DM for 6 d) after seeding, and then cultured in DM containing 30 μM 5-HT for 24 to 96 h (Fig. 1A). 5-HT decreased β-casein mRNA levels in a time-dependent manner, as compared to the control (Fig. 1A).

Subsequently, we quantified the protein levels of β-casein, pSTAT5, and STAT5 by Western blot analysis in samples from cells treated for 72 h with 30 μM 5-HT. The ratio of pSTAT5 to STAT5 (pSTAT5/STAT5) was determined to estimate the phosphorylation status of STAT5. Treatment with 30 μM 5-HT resulted in a significant decrease in β-casein expression and the pSTAT5/STAT5 ratio, as compared to control cells (Fig. 1B).

Expression of 5-HTRs and Tryptophan Hydroxylase 1 in MCF-12A Cells A previous study showed that four 5-HTR isoforms (5-$HTR_{1D,\ 2B,\ 3A,\ and\ 7}$) were expressed in primary human mammary epithelial cells.[17] To investigate the expression levels of these 5-HT isoforms in MCF-12A cells with or without PRL treatment, RT-PCR and Western blot analysis were performed.

mRNA expression of all four 5-HTR subtypes was detectable in untreated MCF-12A cells, and PRL treatment significantly enhanced the expression levels of 5-HTR_{1D}, 5-HTR_{3A}, and 5-HTR_7 mRNA (Fig. 2A). The 5-HTR_{2B} mRNA level was significantly lower than the other receptors, with a relative expression level of 0.0013 in untreated cells; however, it was slightly enhanced by PRL treatment (0.0028; Fig. 2A). In PRL-treated cells, 5-HTR_{1D} and 5-HTR_7 mRNA levels were significantly higher than the other receptors (Fig. 2A). At the protein level, PRL treatment significantly increased the expression of 5-HTR_{1D}, 5-HTR_{3A}, and 5-HTR_7, and slightly increased 5-HTR_{2B} (Fig. 2B). These results suggest that the effects of PRL on 5-HTR_{1D} and 5-HTR_7 expression are important for the suppression of β-casein.

In addition, we investigated the effects of PRL on TPH1 in MCF-12A cells. Mammary epithelial cells activate 5-HT biosynthesis *via* TPH1, which catalyzes the rate-limiting step in 5-HT biosynthesis within the mammary gland during lactation.[7] The mRNA level of TPH1 in MCF-12A cells in the presence or absence of PRL treatment was evaluated by RT-PCR. TPH1 mRNA was detectable in untreated MCF-12A cells, and PRL treatment significantly increased its expression (Fig. 2C).

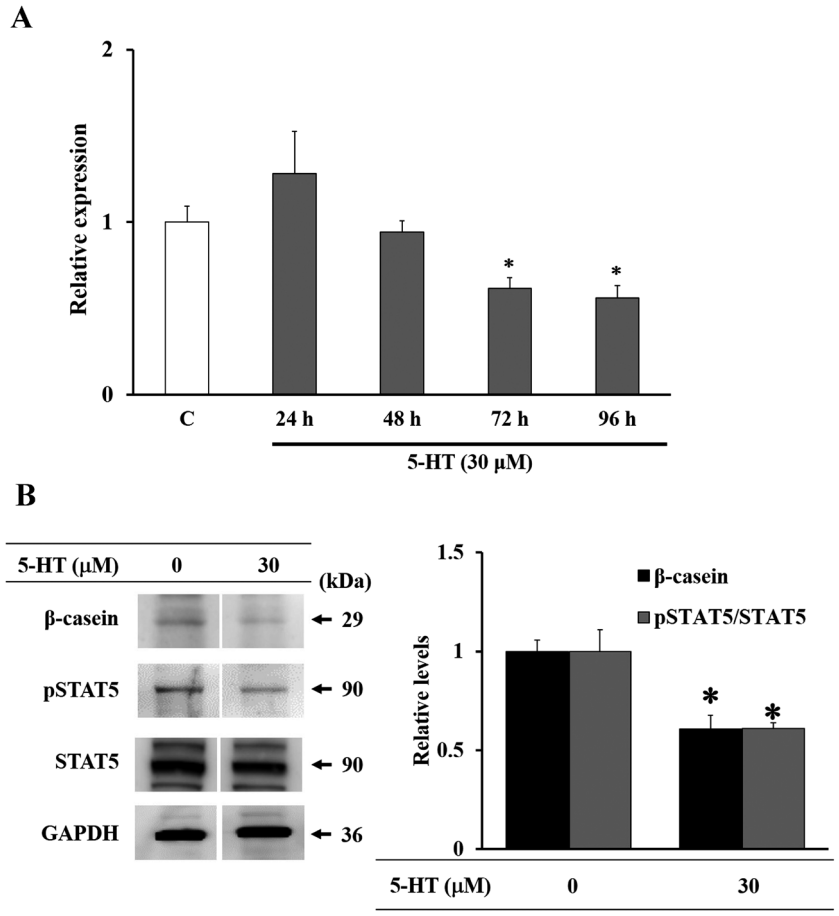

Fig. 1. Effect of 5-HT Treatment on β-Casein, pSTAT5, and STAT5 Expression in MCF-12A Cells

A) The relative mRNA levels of β-casein in cells cultured in the presence or absence of 30 μM 5-HT were analyzed by quantitative RT-PCR relative to an internal standard (*GAPDH*) for the indicated culture times in serum-free DM (containing 0.1 μg/mL PRL). B) The relative protein levels of β-casein and the ratio of pSTAT5 to STAT5 (pSTAT5/STAT5) were analyzed by Western blot in 5-HT-treated and untreated cells. The cells were treated with 30 μM 5-HT for 72 h. Closed and shaded bars indicate the relative levels of β-casein and pSTAT5/STAT5, respectively. The space between the untreated-group (0 μM) and the 30 μM 5-HT-treated group indicates that they were assembled from different areas of the same blots. The values are the means±S.E.M. (n=3–4). *p<0.05, Student's t-test, *versus* untreated cells (0 μM).

Effect of Selective 5-HTR Antagonists on β-Casein Expression in MCF-12A Cells To investigate the possible contribution of 5-HTR_{1D} to the 5-HT-mediated reduction in β-casein in MCF-12A cells, we determined the protein levels of β-casein, pSTAT5, and STAT5 in cells treated with 5-HT (30 μM) and/or a selective 5-HTR_{1D} inhibitor, BRL15572 (30 or 100 μM) for 72 h (Fig. 3A). Treatment with BRL15572 (30 μM or 100 μM) alone had no effect on β-casein expression or the pSTAT5/STAT5 ratio. Further, there were no differences between cells treated with 5-HT alone and cells co-treated with BRL15572 and 5-HT (Fig. 3A).

Similarly, we analyzed the effects of the selective 5-HTR_7 inhibitor, SB269970, on β-casein, pSTAT5, and STAT5 expression in MCF-12A cells treated with 5-HT. Treatment with 100 μM SB260070 slightly increased β-casein and pSTAT5/STAT5 levels, as compared to untreated cells. The reduction in β-casein expression and the pSTAT5/STAT5 ratio was inhibited by SB269970 in a concentration-dependent manner in MCF-12A cells treated with 5-HT. Further, co-treatment with 100 μM SB269970 and 30 μM 5-HT slightly increased β-casein expression and the pSTAT5/STAT5 ratio, compared to untreated cells (Fig. 3B).

DISCUSSION

The objective of this study was to identify the 5-HT receptor subtype involved in the suppression of β-casein expression in response to PRL in human mammary epithelial cells. We previously showed that PRL treatment induced β-casein expression in MCF-12A cells *via* activation of the Jak2/STAT5 pathway. Treatment with 100 μM 5-HT for 72 h inhibited β-casein expression, and this inhibition was associated with the suppression of STAT5 phosphorylation.[15] In this study, we found that 30 μM 5-HT inhibited β-casein mRNA expression in a time-dependent manner (Fig. 1A).

Subsequently, we evaluated the expression of 5-HTR isoforms in MCF-12A cells. A previous study showed that 5-$HTR_{1D, 2B, 3A, and 7}$ mRNAs were present in primary human mammary epithelial cells.[17] Therefore, we evaluated the mRNA and protein levels of these receptor subtypes in MCF-12A cells. We found that all four receptor subtypes expressed at the mRNA and protein level in MCF-12A cells, and that their expression levels were elevated by PRL treatment (Figs. 2A, B). Additionally, the expression of 5-HTR_{1D} and 5-HTR_7 mRNA was significantly higher in PRL-treated cells (Fig. 2A). Since adult mammary gland development is regulated by various hormones, including PRL, during pregnancy, our results may imply that human mammary epithelial cells may

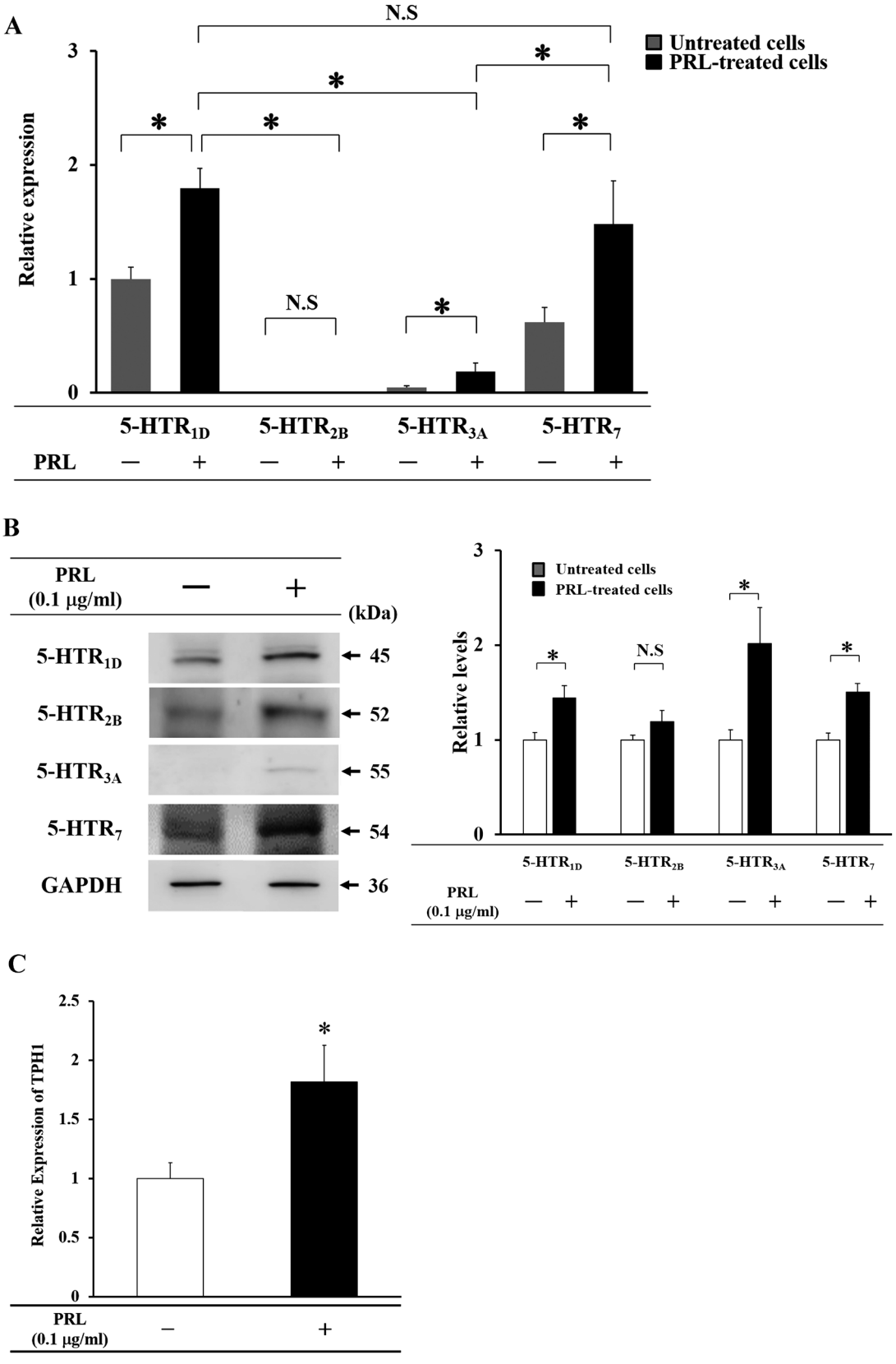

Fig. 2. Expression of 5-HT Receptors and Tryptophan Hydroxylase 1 in MCF-12A Cells

A) The relative mRNA levels of the 5-HTRs (5-HTR_{1D}, 5-HTR_{2B}, 5-HTR_{3A}, and 5-HTR_7) in PRL-treated (closed bars) and untreated (shaded bars) cells were analyzed by quantitative RT-PCR relative to an internal standard (*GAPDH*). PRL-treated cells were incubated in GM after seeding for 24 h, and then grown in serum-free DM (0.1 μg/mL PRL) for 6 d. Untreated cells were grown in GM for 7 d after seeding. The values are the means±S.E.M. (n=3–4). *p<0.05, Tukey–Kramer test. B) The relative protein levels of the 5-HTRs (5-HTR_{1D}, 5-HTR_{2B}, 5-HTR_{3A}, and 5-HTR_7) in PRL-treated (closed bars) and untreated (shaded bars) cells were analyzed by Western blot, respectively. The values are the means±S.E.M. (n=3–4). *p<0.05, Student's t-test. C) The relative mRNA levels of TPH1 in PRL-treated (closed bar) and untreated (open bar) cells were analyzed by quantitative RT-PCR relative to an internal standard (*GAPDH*). PRL-treated cells were incubated in GM after seeding for 24 h, and then grown in serum-free DM (0.1 μg/mL) for 6 d. Untreated cells were grown in GM for 7 d after seeding. The values are the means±S.E.M. (n=3–4). *p<0.05, Student's t-test.

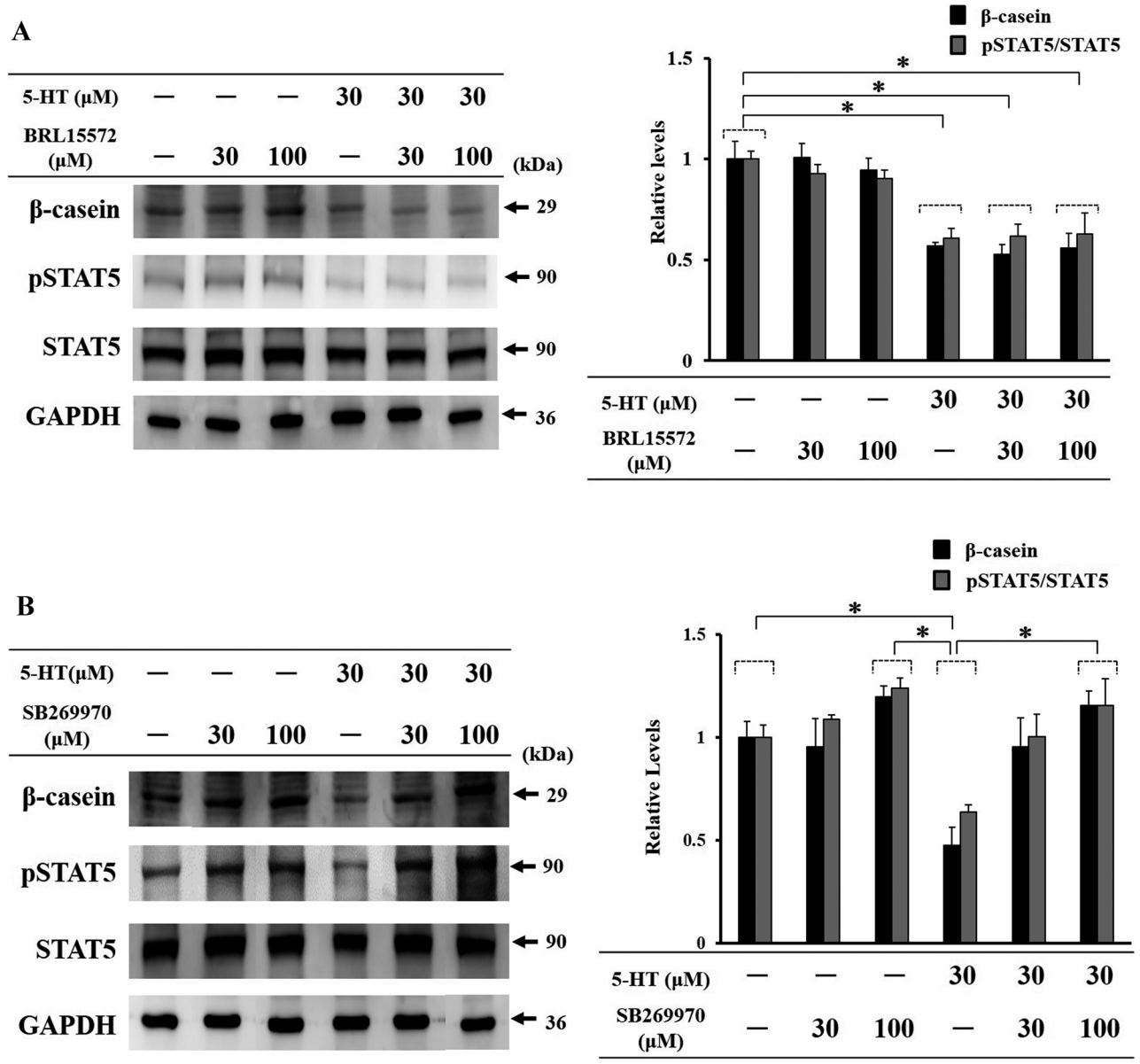

Fig. 3. Effects of Selective 5-HT Receptor Antagonists on β-Casein Expression in MCF-12A Cells

A) The relative protein levels of β-casein, pSTAT5, and STAT5 in cells treated with the indicated concentrations of 5-HT and/or the selective 5-HTR_{1D} antagonist (BRL15572) for 72 h were analyzed by Western blot. Closed bars and shaded bars indicate the relative levels of β-casein and pSTAT5/STAT5, respectively. B) The relative protein levels of β-casein, pSTAT5, and STAT5 in cell extracts treated with the indicated concentrations of 5-HT and/or the selective 5-HTR_7 antagonist (SB269970) for 72 h were analyzed by Western blot. Closed bars and shaded bars indicate the relative levels of β-casein and pSTAT5/STAT5, respectively. The values are the mean±S.E.M. (n=3–4). *$p<0.05$, Tukey–Kramer test.

upregulate 5-HTR expression during lactation, rather than before pregnancy.

We focused on the most highly expressed receptors, 5-HTR_{1D} and 5-HTR_7, and evaluated whether they regulate the suppression of β-casein following 5-HT treatment. β-Casein expression and the pSTAT5/STAT5 ratio were equal in cells co-treated with 5-HT and BRL15572-and cells treated with 5-HT alone (Fig. 3A). However, in cells co-treated with 5-HT and SB269970, the levels of β-casein and pSTAT5/STAT5 were increased in a SB269970 concentration-dependent manner, compared to the cells treated with 5-HT alone (Fig. 3B). These results indicated that the suppression of β-casein was closely associated with 5HT/5-HTR_7 signaling, and that this signaling may be mediated by STAT5 phosphorylation. 5-HTR_7 is a seven-transmembrane-domain Gs-protein-coupled receptor. The coupling between 5-HTR_7 and the Gs-protein results in increased adenylyl cyclase activity leading to the production of cAMP-dependent protein kinase A (PKA).[19] In contrast, PKA is a negative feedback regulator of STAT5-mediated transcription.[20] Although we did not identify a pathway between 5-HTR_7 and STAT5 phosphorylation in this study, β-casein suppression by 5-HT in our model may be related to PKA activation.

In a culture model using bovine mammary epithelial cells, treatment with 5-HTR_{1B}, 5-HTR_{2A}, or 5-HTR_7 agonists increased β-casein mRNA expression.[18] We did not evaluate the expression of 5-HTR_{1B} and 5-HTR_{2A} in MCF-12A cells. Previous work indicated that these 5-HT subtypes were not present in primary human mammary epithelial cells or in an established human mammary epithelial cell line, MCF-10A.[17]

Thus, it is unlikely that 5-HTR_{1B} and 5-HTR_{2B} are associated with 5-HT-mediated suppression of milk protein expression. Additionally, although we did not evaluate the localization of 5-HTR_7 in MCF-12A cells in this study, 5-HTR_7 was localized at the basal and basolateral membrane in MCF-10A cells.[9] MCF-12A cells form poor tight junctions in monolayer culture.[21] Therefore, inhibition of β-casein expression caused by 5-HT in our model may be attributable to 5-HT transfer to the interstitium through the tight junction barrier, resulting in binding of 5-HT to 5-HTR_7 at the basolateral membrane.

Mammary epithelial cells activate 5-HT synthesis through TPH1 during lactation.[7] 5-HT synthesized in mammary glands is released into both the apical (milk) and basolateral space by a vesicular monoamine transporter (VMAT).[8] Our experiments confirmed that MCF-12A cells express TPH1, suggesting that the cells might produce 5-HT and release it into extracellular spaces. Therefore, our observation that treatment with SB269970 (100 μM) alone slightly increased β-casein expression and the pSTAT5/STAT5 ratio, and that co-treatment with 5-HT (30 μM) and SB269970 (100 μM) also increased β-casein expression and the pSTAT5/STAT5 ratio (Fig. 3B), may be due to SB269970-mediated inhibition of binding between endogenous 5-HT and 5-HTR_7. Further studies will be required to determine whether MCF-12A cells synthesize 5-HT, and whether extracellular 5-HT content in PRL-treated MCF-12A cells is elevated compared to untreated cells.

Furthermore, fluoxetine, a selective serotonin transporter inhibitor (SSRI) that perturbs serotonin balance by binding to SERT within the mammary gland, inhibits β-casein expression in primary bovine mammary epithelial cells.[22] To elucidate endogenous 5-HT-mediated mechanisms in MCF-12A cells, it will be important to evaluate not only SSRI action, but also SERT expression.

In conclusion, we showed that 5-HT mediated the suppression of β-casein expression *via* 5-HTR_7 in MCF-12A human mammary epithelial cells.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES

1) Wilde CJ, Addey CV, Boddy LM, Peaker M. Autocrine regulation of milk secretion by a protein in milk. *Biochem. J.*, **305**, 51–58 (1995).
2) Rui H, Lebrun JJ, Kirken RA, Kelly PA, Farrar WL. JAK2 activation and cell proliferation induced by antibody-mediated prolactin receptor dimerization. *Endocrinology*, **135**, 1299–1306 (1994).
3) Clevenger CV, Kline JB. Prolactin receptor signal transduction. *Lupus*, **10**, 706–718 (2001).
4) Liu X, Robinson GW, Hennighausen L. Activation of Stat5a and Stat5b by tyrosine phosphorylation is tightly linked to mammary gland differentiation. *Mol. Endocrinol.*, **10**, 1496–1506 (1996).
5) Wagner KU, Krempler A, Triplett AA, Qi Y, George NM, Zhu J, Rui H. Impaired alveologenesis and maintenance of secretory mammary epithelial cells in Jak2 conditional knockout mice. *Mol. Cell. Biol.*, **24**, 5510–5520 (2004).
6) Lauder JM. A role for serotonin in the mammary gland. *Dev. Cell*, **6**, 165 (2004).
7) Matsuda M, Imaoka T, Vomachka AJ, Gudelsky GA, Hou Z, Mistry M, Bailey JP, Nieport KM, Walther DJ, Bader M, Horseman ND. Serotonin regulates mammary gland development *via* an autocrine–paracrine loop. *Dev. Cell*, **6**, 193–203 (2004).
8) Marshall AM, Hernandez LL, Horseman ND. Serotonin and serotonin transport in the regulation of lactation. *J. Mammary Gland Biol. Neoplasia*, **19**, 139–146 (2014).
9) Stull MA, Pai V, Vomachka AJ, Marshall AM, Jacob GA, Horseman ND. Mammary gland homeostasis employs serotonergic regulation of epithelial tight junctions. *Proc. Natl. Acad. Sci. U.S.A.*, **104**, 16708–16713 (2007).
10) Pai VP, Horseman ND. Biphasic regulation of mammary epithelial resistance by serotonin through activation of multiple pathways. *J. Biol. Chem.*, **283**, 30901–30910 (2008).
11) Pai VP, Horseman ND. Multiple cellular responses to serotonin contribute to epithelial homeostasis. *PLoS ONE*, **6**, e17028 (2011).
12) Pai VP, Marshall AM. Intraluminal volume homeostasis: A common sertonergic mechanism among diverse epithelia. *Commun. Integr. Biol.*, **4**, 532–537 (2011).
13) Hernandez LL, Gregerson KA, Horseman ND. Mammary gland serotonin regulates parathyroid hormone-related protein and other bone-related signals. *Am. J. Physiol. Endocrinol. Metab.*, **302**, E1009–E1015 (2012).
14) Laporta J, Moore SA, Peters MW, Peters TL, Hernandez LL. Short communication: Circulating serotonin (5-HT) concentrations on day 1 of lactation as a potential predictor of transition-related disorders. *J. Dairy Sci.*, **96**, 5146–5150 (2013).
15) Chiba T, Kimura S, Takahashi K, Morimoto Y, Sanbe A, Ueda H, Kudo K. Serotonin suppresses β-casein expression *via* inhibition of signal transducer and activator of transcription 5 (STAT5) protein phosphorylation in human mammary epithelial cells MCF-12A. *Biol. Pharm. Bull.*, **37**, 1336–1340 (2014).
16) Hannon J, Hoyer D. Molecular biology of 5-HT receptors. *Behav. Brain Res.*, **195**, 198–213 (2008).
17) Pai VP, Marshall AM, Hernandez LL, Buckley AR, Horseman ND. Altered serotonin physiology in human breast cancers favors paradoxical growth and cell survival. *Breast Cancer Res.*, **11**, R81 (2009).
18) Hernandez LL, Limesand SW, Collier JL, Horseman ND, Collier RJ. The bovine mammary gland expresses multiple functional isoforms of serotonin receptors. *J. Endocrinol.*, **203**, 123–131 (2009).
19) Ling L, Lobie PE. RhoA/ROCK activation by growth hormone abrogates p300/histone deacetylase 6 repression of Stat5-mediated transcription. *J. Biol. Chem.*, **279**, 32737–32750 (2004).
20) Guseva D, Wirth A, Ponimaskin E. Cellular mechanisms of the 5-HT7 receptor-mediated signaling. *Front. Behav. Neurosci.*, **8**, 306 (2014).
21) Zhang Y, Zhang B. D4-GDI, a Rho GTPase regulator, promotes breast cancer cell invasiveness. *Cancer Res.*, **66**, 5592–5598 (2006).
22) Hernandez LL, Collier JL, Vomachka AJ, Collier RJ, Horseman ND. Suppression of lactation and acceleration of involution in the bovine mammary gland by a selective serotonin reuptake inhibitor. *J. Endocrinol.*, **209**, 45–54 (2011).