Placenta 32 (2011) 651–656

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Stimulation of serotonergic 5-HT_{2A} receptor signaling increases placental aromatase (CYP19) activity and expression in BeWo and JEG-3 human choriocarcinoma cells

T. Klempan, A.-A. Hudon-Thibeault, T. Oufkir, C. Vaillancourt*, J.T. Sanderson*

INRS-Institut Armand-Frappier, Université du Québec, 531, boulevard des Prairies, Building 18, Laval, QC, H7V 1B7 Canada

ARTICLE INFO

Article history:
Accepted 6 June 2011

Keywords:
Serotonin
5-HT_{2A} receptor
Placental aromatase (CYP19)
Estrogens
Gene regulation
Human trophoblast
JEG-3
BeWo
Protein kinase C (PKC)

ABSTRACT

It is known that serotonin can influence the production and function of sex hormones, such as estrogens. Estrogens are critical for maintenance of pregnancy and regulate placental and fetal development. The key enzyme controlling estrogens synthesis during pregnancy is placental aromatase (CYP19). To better understand the regulation of placental aromatase, this study determined whether serotonin is involved in the regulation of this enzyme. BeWo and JEG-3 choriocarcinoma cells were used as models of the human placental trophoblast to evaluate the effects of serotonin and selective 5-HT_{2A} receptor agonists on CYP19 activity and expression. Serotonin and selective 5-HT_{2A} receptor agonists as well as PKC activation increased aromatase activity and expression in BeWo and JEG-3 cells. Dexamethasone, which regulates aromatase expression via JAK/STAT activation in certain tissues, had no effect. Increased *CYP19* gene transcription by 5-HT_{2A} receptor and PKC stimulation was mediated by activation of the placental I.1 aromatase promoter. This study shows that the serotonergic system modulates placental aromatase expression, which would result in altered estrogens biosynthesis in trophoblast cells. Future detailed studies of serotonin–estrogen interactions in placenta are crucial for an improved understanding of the endo-, para- and autocrine role of serotonin during pregnancy and fetal development.

1. Introduction

In humans, placental estrogens regulate various functions critical for the maintenance of healthy pregnancy and fetal development, such as trophoblast differentiation/invasion, uterine growth, and progesterone synthesis [1–3]. Placental estrogen synthesis is catalyzed by the rate-limiting enzyme aromatase (CYP19) [4]. *CYP19* is expressed predominantly in differentiated syncytiotrophoblast cells as well as in JEG-3 and BeWo human choriocarcinoma cell lines [5–7]. Human placenta lacks steroid 17α-hydroxylase/17, 20-lyase (CYP17) and cannot produce estrogens *de novo* from cholesterol; it is thus dependent on fetal and maternal androgen precursors to produce estrogens and placental aromatase is key in this process. Aromatase gene expression undergoes complex regulation by a number of discrete promoters found in exon I of the *CYP19* gene which are differentially active in various tissues and cell types, with the I.1 promoter predominantly utilized in placenta, and with a minor contribution from the placenta-specific promoters I.2 [8] and recently discovered 1.8 [9]. The placental I.1 aromatase promoter is localized 93 kb upstream of the coding region exon II-X of the *CYP19* gene and its activation produces *CYP19* transcript with exon I.1 spliced onto a common splice junction directly upstream from exon II [10]. The regulation of placental estrogens synthesis by aromatase in the trophoblast is known to be increased by factors including calcitriol [11], hGCMa, a mammalian homolog of the protein encoded by the *Drosophila* glial cells missing (*gcm*) gene [12], normoxic reduction of inhibitory factor Mash-2 [13], and the AP-2 family of transcription factors (through the second messenger cAMP) [12], as well as activators of the protein kinase C (PKC) signaling pathway [14,15]. The precise role of the PKC signaling pathway in the regulation of *CYP19* gene expression remains to be clarified.

We have recently demonstrated that *de novo* synthesis of serotonin (5-hydroxytryptamine; 5-HT) occurs in the human trophoblast cell [16], suggesting an important endo-, para- and autocrine role for serotonin in placental function. This is supported by our earlier finding that serotonin acts via the 5-HT_{2A} receptor as a key regulator of trophoblast growth, a critical cell process involved in placentation [17]. We observed this mitogenic effect of serotonin in

* Corresponding authors. Tel.: +1 450 687 5010; fax: +1 450 686 5309.
E-mail addresses: cathy.vaillancourt@iaf.inrs.ca (C. Vaillancourt), thomas.sanderson@iaf.inrs.ca (J.T. Sanderson).

0143-4004/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.placenta.2011.06.003

BeWo and JEG-3 cells which involved 5-HT_{2A}-mediated stimulation of both the JAK2/STAT3 and PLCβ-PKC-β-Ras-ERK1/2 pathways [17]. It has been reported that activation of 5-HT_{2A} receptor signaling stimulates the 17β-estradiol-mediated secretion of prolactin in dopaminergic neurons of ovariectomized rats, an effect likely due to increased local generation of estrogens by brain aromatase [18]. Moreover, a paracrine control of steroidogenesis by serotonin has been observed in the human adrenal cortex, where it stimulates aldosterone release [19]. In Japanese Medaka exposed to the selective serotonin-reuptake inhibitor (SSRI) fluoxetine increased plasma estradiol levels were observed, although a role for serotonin was not directly confirmed [20]. However, the potential effects of serotonin and 5-HT_{2A} signaling on placental aromatase have not been studied.

Nothing is known about the potential interactions between serotonin and estrogens in human placenta, although neuroendocrinological research in brain has demonstrated that the two hormonal systems are intricately interdependent and interactive. Also, serotonin acts via the placental 5-HT_{2A} receptor to stimulate the PKC signaling pathway, which is known to be involved in the regulation of placental aromatase [15,17,21]. Little is known about the molecular mechanism underlying the ability of serotonin to interact with estrogens biosynthesis. Thus, the objective of this study was to determine how serotonergic stimulation affects the expression and catalytic activity of placental aromatase using human BeWo and JEG-3 choriocarcinoma cell lines as trophoblast models.

2. Materials and methods

2.1. Chemicals

Serotonin (5-HT hydrochloride), the selective 5-HT_{2A} receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) and antagonist ritanserin, dexamethasone (DEX), PKC activator phorbol-12-myristate-13-acetate (PMA), the irreversible aromatase inhibitor 4-hydroxyandrostenedione (formestane), and PKC inhibitor chelerythrine chloride were obtained from Sigma–Aldrich (Oakville, ON). The high affinity 5-HT_{2A} receptor agonist TCB-2 and selective PKC inhibitors GF109203X (also known as Gö6850 or bisindolylmaleimide I) and Gö6976 were obtained from Tocris Bioscience (Ellisville, MO).

2.2. Cell culture

The human placental choriocarcinoma cell lines JEG-3 and BeWo were obtained from the American Type Culture Collection (Rockville, MD). BeWo cells were maintained in MEM/Ham's F-12K (50:50 v/v) culture medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin. JEG-3 cells were cultured in MEM Eagle medium supplemented with 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/mL penicillin and 100 mg/mL streptomycin. Cells were maintained in 75 cm^2 filter-cap culture flasks (Techno Plastic Products, MIDSCI, St-Louis, MO) in a humidified atmosphere with 5% CO_2 at 37 °C. Cells were trypsinized (0.5% trypsin) and transferred to new 75 cm^2 flasks when they reached 90% confluence. For aromatase experiments, cells were treated as described previously [22,23]. Briefly, JEG-3 (2.5×10^4 cells/well) and BeWo (5×10^4 cells/well) cells were plated in 24-well culture plates containing 1 mL medium per well and exposed to various concentrations of the test compounds in aqueous solution or DMSO (0.2% final concentration in culture medium).

2.3. Aromatase catalytic activity

The catalytic activity of aromatase was determined by a tritiated water release assay according to the method of Lephart and Simpson [24] with certain modifications [23,25]. JEG-3 and BeWo cells were exposed to 54 nM 1β-^{3}H-androstenedione (Perkin Elmer, Wellesley, MA) in serum-free culture medium and incubated for 1.5 h at 37 °C in an atmosphere with 5% CO_2. 4-Hydroxyandrostenedione was used as a positive control for inhibition of aromatase activity to verify the specificity of the water release assay in both cell lines. A cell-free control using only medium was included in each experiment to correct for enzyme-independent tritiated water release. Further steps were as reported previously [22,23]. Aromatase activities (pmole/min/mg cellular protein) were expressed as percent of control (DMSO) activity.

2.4. Expression of CYP19 mRNA

RNA was isolated following 24 h exposures to the test compounds (Table 1) using the High Pure RNA Isolation Kit (Roche Diagnostics, Mississauga, ON) according to manufacturer instructions. Concentration and purity of RNA was determined by measuring the 260/280 nm absorbance ratio using a spectrophotometer (SpectraMax M5, Molecular Devices, Sunnyvale, CA), with quality assessed visually from 18S and 28S ribosomal bands on agarose gels. Original extracts and 50 ng/μl dilutions in RNAase-free water were stored at −80 °C until RT-PCR analysis.

Primers that recognized either the common coding region (exon II-X) of aromatase [23] or placental I.1-promoter-derived [26,27] *CYP19* transcripts were used for amplification (Access RT-PCR kit, Promega, Madison, WI) of cellular total RNA preparations, using primers for β-actin as reference gene. Conditions for *CYP19* mRNA amplification were: 100 ng RNA, 0.75 mM Mg^{2+}, annealing temperature 57 °C (exon II-X) or 61 °C (I.1), for 35 and 30 cycles, respectively. For β-actin amplification conditions were: 10 ng RNA, 2 mM Mg^{2+}, annealing temperature 54 °C for 25 cycles. Primer sequences were 5′-TTA-TGA-GAG-CAT-GCG-GTA-CC-3′ (fwd) and 5′-CTT-GCA-ATG-TCT-TCA-CGT-GG-3′ (rev) for the 314 bp exon II-X *CYP19* product, 5′-GGA-TCT-TCC-AGA-CGT-CGC-GA-3′ (fwd) and 5′-CAT-GGC-TTC-AGG-CAC-GAT-GC-3′ (rev) for the 119 bp I.1 *CYP19* product, and 5′-AAA-CTA-CCT-TCA-ACT-CCA-TC-3′ (fwd) and 5′-ATG-ATC-TTG-ATC-TTC-ATT-GT-3′ (rev) for the 163 bp β-actin product. Amplification products were separated and detected on 2% agarose gels stained with ethidium bromide. Densitometry was carried out using AlphaEaseFC Imaging software (version 6.0.0, Alpha Innotech, San Leandro, CA) and normalized to β-actin. The exponential amplification range was determined for each set of primers to optimize the number of cycles in the PCR method for semi-quantitative analysis of gene products, as done previously [23,28]. Exposure experiments were performed three times and amplification reactions were performed in triplicate per experiment.

2.5. Statistical analyses

Experiments were performed three times; per experiment each treatment was performed in triplicate. Statistically significant differences ($P < 0.05$) were determined by Student *t*-test or one-way ANOVA followed by Tukey post-hoc test or Dunnett's post-hoc test for multiple comparisons to control (GraphPad Prism v.5.04, GraphPad Software, San Diego, CA).

3. Results

3.1. Serotonin and 5-HT_{2A} receptor agonists stimulate aromatase activity and expression

Serotonin increased aromatase activity concentration-dependently (1–30 μM) in BeWo, but not JEG-3 cells (Fig. 1A). The selective 5-HT_{2A} receptor agonists DOI (10 μM) and TCB-2 (300 μM) led to a statistically significant increase in aromatase activity in both JEG-3 and BeWo cells (1.2- and 2.0-fold, respectively for DOI, and 1.6 and 2.1-fold, respectively, for TCB-2) (Fig. 1B). These increases were greater than those seen with serotonin. The positive control PMA increased aromatase activity in JEG-3 and BeWo cells by 1.4 and 3.0-fold, respectively (Fig. 1C). Levels of I.1 promoter-derived and promoter non-specific (exon II-X) *CYP19* mRNA were increased statistically significantly by serotonin in BeWo

Table 1
Effects of serotonin, selective 5-HT_{2A} receptor agonist (DOI), dexamethasone (DEX) and phorbol-12-myristate-13-acetate (PMA) on semi-quantitative I.1 promoter- or promoter non-specific- (exon II-X) CYP19 expression in BeWo and JEG-3 cells after a 24 h exposure. *Statistically significant difference from DMSO control ($P < 0.05$, one-way ANOVA with Tukey post-hoc test).

	Treatment	JEG-3	BeWo
		Mean ± SD	Mean ± SD
I.1	DMSO	1.00 ± 0.15	1.00 ± 0.21
	30 μM Serotonin	1.35 ± 0.20	1.46 ± 0.20*
	30 μM DOI	1.41 ± 0.23*	1.61 ± 0.24*
	1 μM DEX	1.13 ± 0.11	1.14 ± 0.12
	1 μM PMA	1.48 ± 0.17*	2.53 ± 0.06*
Exon II-X	DMSO	1.00 ± 0.14	1.00 ± 0.18
	30 μM Serotonin	1.31 ± 0.16	1.44 ± 0.22*
	30 μM DOI	1.43 ± 0.27*	1.56 ± 0.14*
	1 μM DEX	1.02 ± 0.12	1.27 ± 0.25
	1 μM PMA	1.53 ± 0.12*	2.51 ± 0.10*

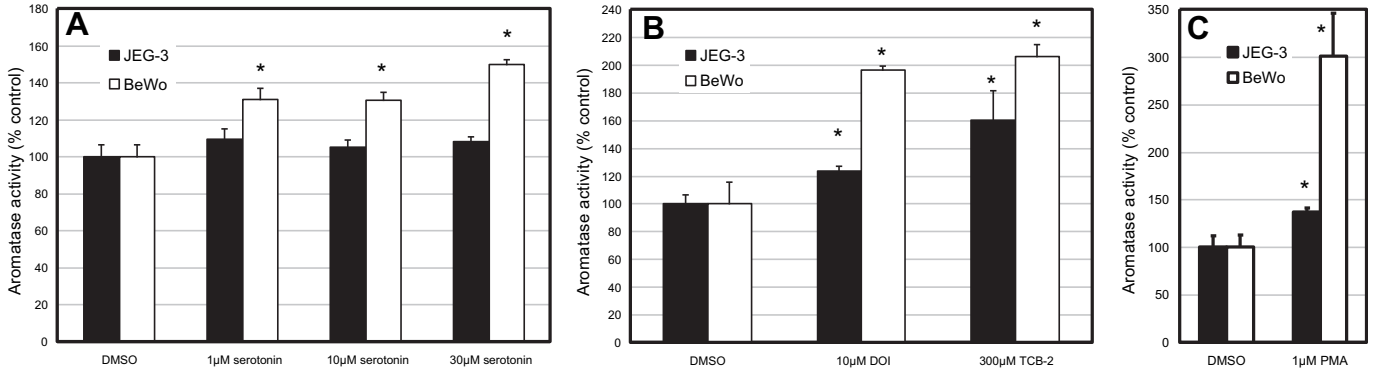

Fig. 1. Aromatase activity (mean ± SD) in JEG-3 and BeWo cells treated with (A) serotonin, (B) 5-HT_{2A} agonists DOI or TCB-2 and (C) protein kinase C (PKC) activator phorbol-12-myristate-13-acetate (PMA). Serotonin increased aromatase activity statistically significantly above DMSO control in BeWo cells (*$P < 0.05$, one-way ANOVA with Tukey post-hoc test); DOI, TCB-2 and PMA significantly induced aromatase activity in both cell lines (*$P < 0.05$, Student *t*-test). Exposures were for 24 h in quadruplicate.

(1.4–1.5-fold), but not JEG-3 cells (Table 1). DOI (10 μM) increased *CYP19* transcript levels significantly in both JEG-3 (about 1.4 fold) and BeWo (about 1.6 fold) cell lines. The positive control PMA increased *CYP19* mRNA expression in JEG-3 and BeWo cells by about 1.5 and 2.5-fold, respectively. Dexamethasone (DEX, 1 μM) did not alter *CYP19* expression levels in JEG-3 or BeWo cells (Table 1) and the same was observed for aromatase catalytic activity (not shown). None of the treatments affected JEG-3 or BeWo cell proliferation after a 24 h exposure (not shown), consistent with our previous findings [17]. A detailed concentration-response experiment in BeWo cells showed that serotonin caused a statistically significantly increase in aromatase activity at concentrations of 0.1 μM and above, with activity increased 1.5-fold at the highest tested concentration of 30 μM (Fig. 2). Serotonin-, DOI- and TCB-2-mediated induction of aromatase activity in BeWo cells was abolished by 10 μM ritanserin, a potent, selective 5-HT_{2A} receptor antagonist (Fig. 3).

3.2. Involvement of PKC signaling in serotonergic regulation of *CYP19* expression

PMA, a known activator of the PKC signaling pathway which is involved in the regulation of placental aromatase, increased aromatase activity in BeWo cells about 3–4-fold (Fig. 4), an effect that was blocked concentration-dependently by the highly selective PKC-α/β1 inhibitors GF109203X (30–200 nM) or Gö6976 (100 nM) (Fig. 4). Chelerythrin, a less potent and less selective PKC inhibitor, decreased PMA-mediated aromatase induction at a concentration of 1 μM, but not 300 nM (Fig. 4). GF109203X (30 nM) and Gö6976 (100 nM) also decreased DOI-mediated induction of aromatase activity in BeWo cells (Fig. 5). The 3–4-fold lesser PKC-α/β1 inhibitory potencies of GF109203X (IC_{50} = 0.008/0.018 μM) compared with Gö6976 (IC_{50} = 0.002/0.006 μM) [29] were reflected in our experiments (Figs. 4 and 5).

4. Discussion

This study is the first to show that serotonergic stimulation in human placental trophoblast models increases aromatase expression and catalytic activity, and that this effect is mediated, in part, via the 5-HT_{2A} receptor and activation of the PKC pathway. Aromatase activity and *CYP19* gene expression were elevated following exposure of JEG-3 and BeWo choriocarcinoma cells to either serotonin or the 5-HT_{2A} receptor-selective agonists DOI and TCB-2, effects that were blocked by the selective 5-HT_{2A} receptor antagonist ritanserin. Serotonergic stimulation of aromatase was considerably more pronounced in BeWo than JEG-3 cells, a difference in responsiveness that may be explained by differences in the regulatory environment controlling aromatase expression between these cell lines [30]. BeWo and JEG-3 cells possess different characteristics and do not always respond similarly to various

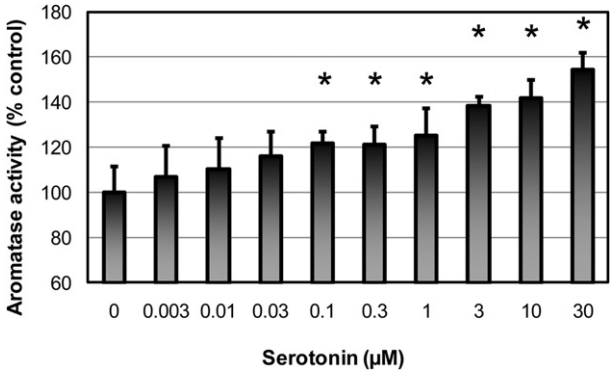

Fig. 2. Concentration-dependent induction of aromatase activity by serotonin in BeWo cells. Aromatase activity was increased significantly compared to DMSO control (0 μM serotonin) at concentrations greater than 0.1 μM (*$P < 0.05$, one-way ANOVA and Dunnett post-hoc test for multiple comparisons). Exposures were for 24 h in quadruplicate.

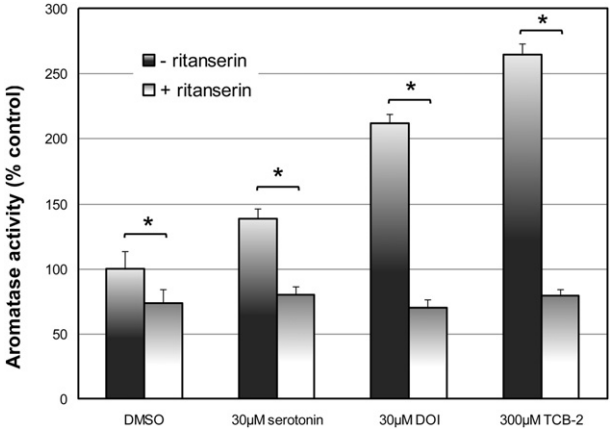

Fig. 3. The 5-HT_{2A} receptor antagonist ritanserin significantly reduced DOI-induced aromatase activity in BeWo cells compared to the respective treatment without ritanserin (*$P < 0.05$, Student *t*-test). Exposures were for 24 h in quadruplicate. Activities are expressed as % of DMSO control without ritanserin.

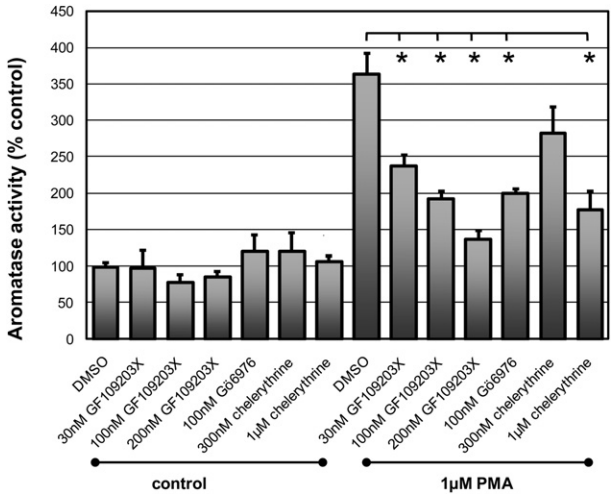

Fig. 4. Effects of selective protein kinase C (PKC) inhibitors, GF109203X, Gö6976 and chelerythrine, on phorbol-12-myristate-13-acetate (PMA)-stimulated aromatase activity in BeWo cells. *Significantly different from PMA treatment alone (*$P < 0.05$, Student *t*-test). Exposures were for 24 h in quadruplicate. Activities are expressed as % of untreated control (DMSO).

physiological or pharmacological stimuli [31,32]. Under our conditions, basal aromatase activity in JEG-3 cells was about 15–20 pmol/h/mg cellular protein and at least 10-fold greater than that in BeWo cells (1–2 pmol/h/mg cellular protein), which is consistent with other reports [33]. It is possible that basal serotonin levels in FBS (<0.5 μM) may have attenuated the response to further serotonin exposure in JEG-3 cells, although this would likely only result in a greater basal activity of aromatase and slight shift of the concentration-response curve. Semi-quantitatively determined expression levels of I.1 promoter-derived *CYP19* mRNA were about 4–5-fold greater in JEG-3 than BeWo cells in our experiments (not shown), which is consistent with a microarray study of differences in gene expression, including *CYP19*, between JEG-3 and BeWo cells [34]. This indicates that *CYP19* promoter activity is constitutively more active in JEG-3 than BeWo cells possibly leaving less room for further stimulation via the PKC pathway. Also, PKC signaling may vary in its effectiveness to induce aromatase expression in JEG-3 and BeWo cells through alternate usage of *CYP19* promoters,

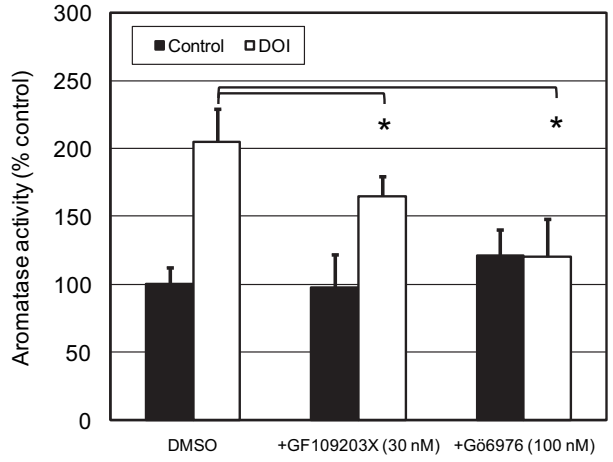

Fig. 5. Effects of selective protein kinase C (PKC) inhibitors, GF109203X and Gö6976 on DOI-stimulated aromatase activity in BeWo cells. *Significantly different from DOI treatment alone (*$P < 0.05$, Student *t*-test). Exposures were for 24 h in quadruplicate. Activities are expressed as % of untreated control (DMSO).

possibly due to selective silencing or recruitment of *CYP19* promoters, structural alterations in certain promoter regions, or other unique differences in gene transactivation mechanisms [6,10,11,13,15,35–39].

Consistent with our findings in BeWo cells, phorbol esters increased expression and activity of placental aromatase in JAR human placental choriocarcinoma cells through a mechanism involving upregulation of *CYP19* mRNA synthesis [40]. An additional mechanism of serotonin-mediated induction of placental aromatase in BeWo cells could be via 5-HT_{2A} receptor-specific activation of the JAK2/STAT3 pathway, which we have recently shown to be involved in serotonin-stimulated BeWo cell growth [17]. The known impact of serotonin on JAK2/STAT3 activation in vascular smooth muscle [41], and the role of JAK/STAT activation in desensitization of 5-HT_{2A} receptor-stimulated PLC signaling by olanzapine and clozapine (or inhibition of signaling by the 5-HT_{2A} antagonist MDL 100907) [42] suggest that this pathway may modulate regulation of placental aromatase expression. More directly, serotonergic JAK2/STAT3 stimulation may result in aromatase induction via the placenta-specific 1.8 *CYP19* promoter which, although a minor contributor to total placental *CYP19* expression, is known to be glucocorticoid-responsive [9]. Furthermore, in adipose tissue aromatase is regulated via a glucocorticoid-responsive I.4 promoter which also involves activation of the JAK/STAT signaling pathway [43]. Although dexamethasone did not cause a statistically significant increase in *CYP19* mRNA levels or aromatase activity in the present study, effects on I.8-mediated aromatase expression were not determined. The effect of selective JAK2 and STAT3 activation and inhibition on 5-HT_{2A}-mediated activation of I.8 promoter-mediated placental aromatase is currently under investigation in various trophoblast models, including placental cells in primary culture.

Our observed serotonergic induction of CYP19 in placental cell lines suggests an important role for the serotonergic system in the control of estrogen biosynthesis during progression of pregnancy, with altered levels of serotonin in the trophoblast potentially leading to impaired placental estrogen production. This implied connection between serotonin and estrogens forms the basis for our general hypothesis that altered serotonergic signaling affects local placental estrogen production, causing disruption of trophoblast development and function with negative consequences for placental and fetal health. The importance of placental serotonin during fetal development has recently been underlined in a study showing that placental serotonin contributes significantly to the modulation of fetal forebrain development in mice [44]. A breakdown in the placental communication between serotonin and estrogens may be responsible for premature losses and other complications that arise during pregnancies accompanied by abuse of drugs such as cocaine, or intake of medications such as SSRIs, which act on the serotonergic system. These pregnant women are at greater risk of premature delivery, preterm onset of labor, and newborns with low birth weight [45–47]. The increased risk of developing obstetric complications associated with altered serotonin function, such preeclampsia [48] and gestational diabetes [49], may also be due to serotonin-mediated disruption of estrogen synthesis and function.

We have shown that serotonin regulates placental aromatase expression through the placenta-specific distal I.1 promoter of the *CYP19* gene. We further show that 5-HT_{2A} receptor stimulation increases aromatase activity and expression via the activation of PKC, an effect also observed with phorbol ester-induced PKC stimulation (most prominently in BeWo cells). BeWo cells are a well established model of the human placental trophoblast and a prime choice of cell line for novel mechanistic studies of trophoblast function [6,7,33]. In this study we have found that aromatase in

BeWo cells responds similarly to the human placental trophoblast [39] to known stimulants of placental aromatase and utilizes the same placenta-specific I.1 aromatase promoter. Our observations are currently being confirmed in primary trophoblast cells, but this present *in vitro* study suggests that placental trophoblastic BeWo cells provide useful *in vitro* models for fundamental and applied studies of serotonin–estrogen interactions at the molecular, biochemical and cellular level. A better understanding of the relationship between serotonin and estrogens is vital for the development of preventive strategies with regard to placental health, and consequently that of mother and developing fetus.

Acknowledgments

We are grateful to Kathy Deroy and Jihane Gasmi for their technical assistance. This study was supported financially by a grant from the Canadian Institutes of Health Research (CIHR, IGO 93972) awarded equally to Thomas Sanderson and Cathy Vaillancourt, as well as a Fondation Armand-Frappier postdoctoral fellowship to Timothy Klempan and a Natural Sciences and Engineering Research Council of Canada (NSERC) studentship to Andrée-Anne Hudon-Thibeault.

References

[1] Bonagura TW, Pepe GJ, Enders AC, Albrecht ED. Suppression of extravillous trophoblast vascular endothelial growth factor expression and uterine spiral artery invasion by estrogen during early baboon pregnancy. Endocrinology 2008;149(10):5078–87.
[2] Yashwanth R, Rama S, Anbalagan M, Rao AJ. Role of estrogen in regulation of cellular differentiation: a study using human placental and rat Leydig cells. Mol Cell Endocrinol 2006;246(1–2):114–20.
[3] Bukovsky A, Cekanova M, Caudle MR, Wimalasena J, Foster JS, Henley DC, et al. Expression and localization of estrogen receptor-alpha protein in normal and abnormal term placentae and stimulation of trophoblast differentiation by estradiol. Reprod Biol Endocrinol 2003;1:13.
[4] Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, et al. The P450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names of enzymes, and nomenclature. DNA Cell Biol 1993;12(1):1–51.
[5] Fournet-Dulguerov N, MacLusky NJ, Leranth CZ, Todd R, Mendelson CR, Simpson ER, et al. Immunohistochemical localization of aromatase cytochrome P-450 and estradiol dehydrogenase in the syncytiotrophoblast of the human placenta. J Clin Endocrinol Metab 1987;65(4):757–64.
[6] Wu X, Iguchi T, Itoh N, Okamoto K, Takagi T, Tanaka K, et al. Ascorbic acid transported by sodium-dependent vitamin C transporter 2 stimulates steroidogenesis in human choriocarcinoma cells. Endocrinology 2008;149(1):73–83.
[7] Bahn RS, Worsham A, Speeg Jr KV, Ascoli M, Rabin D. Characterization of steroid production in cultured human choriocarcinoma cells. J Clin Endocrinol Metab 1981;52(3):447–50.
[8] Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, et al. Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. Endocr Rev 1994;15(3):342–55.
[9] Demura M, Reierstad S, Innes JE, Bulun SE. Novel promoter I.8 and promoter usage in the CYP19 (aromatase) gene. Reprod Sci 2008;15(10):1044–53.
[10] Mendelson CR, Kamat A. Mechanisms in the regulation of aromatase in developing ovary and placenta. J Steroid Biochem Mol Biol 2007;106(1–5):62–70.
[11] Barrera D, Avila E, Hernandez G, Halhali A, Biruete B, Larrea F, et al. Estradiol and progesterone synthesis in human placenta is stimulated by calcitriol. J Steroid Biochem Mol Biol 2007;103(3–5):529–32.
[12] Yamada K, Ogawa H, Honda S, Harada N, Okazaki T. A GCM motif protein is involved in placenta-specific expression of human aromatase gene. J Biol Chem 1999;274(45):32279–86.
[13] Jiang B, Kamat A, Mendelson CR. Hypoxia prevents induction of aromatase expression in human trophoblast cells in culture: potential inhibitory role of the hypoxia-inducible transcription factor Mash-2 (mammalian achaete-scute homologous protein-2). Mol Endocrinol 2000;14(10):1661–73.
[14] Ritvos O, Voutilainen R. Regulation of aromatase cytochrome P-450 and 17 beta-hydroxysteroid dehydrogenase messenger ribonucleic acid levels in choriocarcinoma cells. Endocrinology 1992;130(1):61–7.
[15] Harada N, Yoshimura N, Honda S. Unique regulation of expression of human aromatase in the placenta. J Steroid Biochem Mol Biol 2003;86(3–5):327–34.
[16] Deroy K, St-Pierre J, Sanderson J, Vaillancourt C. Human placental trophoblasts produce serotonin de novo. In: International serotonin club conference, Serotonin: The New Wave New Developments in Serotonin Research; 2010.
[17] Oufkir T, Arseneault M, Sanderson JT, Vaillancourt C. The 5-HT 2A serotonin receptor enhances cell viability, affects cell cycle progression and activates MEK-ERK1/2 and JAK2-STAT3 signalling pathways in human choriocarcinoma cell lines. Placenta 2010;31(5):439–47.
[18] Liang SL, Pan JT. An endogenous serotonergic rhythm acting on 5-HT(2A) receptors may be involved in the diurnal changes in tuberoinfundibular dopaminergic neuronal activity and prolactin secretion in female rats. Neuroendocrinology 2000;72(1):11–9.
[19] Lefebvre H, Compagnon P, Contesse V, Delarue C, Thuillez C, Vaudry H, et al. Production and metabolism of serotonin (5-HT) by the human adrenal cortex: paracrine stimulation of aldosterone secretion by 5-HT. J Clin Endocrinol Metab 2001;86(10):5001–7.
[20] Foran CM, Weston J, Slattery M, Brooks BW, Huggett DB. Reproductive assessment of Japanese medaka (Oryzias latipes) following a four-week fluoxetine (SSRI) exposure. Arch Environ Contam Toxicol 2004;46(4):511–7.
[21] Yang R, You X, Tang X, Gao L, Ni X. Corticotropin-releasing hormone inhibits progesterone production in cultured human placental trophoblasts. J Mol Endocrinol 2006;37(3):533–40.
[22] Sanderson T, Renaud M, Scholten D, Nijmeijer S, van den Berg M, Cowell S, et al. Effects of lactone derivatives on aromatase (CYP19) activity in H295R human adrenocortical and (anti)androgenicity in transfected LNCaP human prostate cancer cells. Eur J Pharmacol 2008;593(1–3):92–8.
[23] Sanderson JT, Seinen W, Giesy JP, van den Berg M. 2-Chloro-s-triazine herbicides induce aromatase (CYP19) activity in H295R human adrenocortical carcinoma cells: a novel mechanism for estrogenicity? Toxicol Sci 2000;54(1):121–7.
[24] Lephart ED, Simpson ER. Assay of aromatase activity. Meth Enzymol 1991;206:477–83.
[25] Sanderson JT, Letcher RJ, Heneweer M, Giesy JP, van den Berg M. Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin production in male carp hepatocytes. Environ Health Perspect 2001;109(10):1027–31.
[26] Kamat A, Alcorn JL, Kunczt C, Mendelson CR. Characterization of the regulatory regions of the human aromatase (P450arom) gene involved in placenta-specific expression. Mol Endocrinol 1998;12(11):1764–77.
[27] Bulun SE, Sebastian S, Takayama K, Suzuki T, Sasano H, Shozu M. The human CYP19 (aromatase P450) gene: update on physiologic roles and genomic organization of promoters. J Steroid Biochem Mol Biol 2003;86(3–5):219–24.
[28] Sanderson JT, Hordijk J, Denison MS, Springsteel MF, Nantz MH, Van Den Berg M. Induction and inhibition of aromatase (CYP19) activity by natural and synthetic flavonoid compounds in H295R human adrenocortical carcinoma cells. Toxicol Sci 2004;82:70–9.
[29] Martiny-Baron G, Kazanietz MG, Mischak H, Blumberg PM, Kochs G, Hug H, et al. Selective inhibition of protein kinase C isozymes by the indolocarbazole Go 6976. J Biol Chem 1993;268(13):9194–7.
[30] Al-Nasiry S, Spitz B, Hanssens M, Luyten C, Pijnenborg R. Differential effects of inducers of syncytialization and apoptosis on BeWo and JEG-3 choriocarcinoma cells. Hum Reprod 2006;21(1):193–201.
[31] Bilban M, Barth S, Cervar M, Mauschitz R, Schaur RJ, Zivkovic F, et al. Differential regulation of endothelin secretion and endothelin receptor mRNA levels in JAR, JEG-3, and BeWo choriocarcinoma cell lines and in human trophoblasts, their nonmalignant counterpart. Arch Biochem Biophys 2000;382(2):245–52.
[32] Hohn HP, Linke M, Ugele B, Denker HW. Differentiation markers and invasiveness: discordant regulation in normal trophoblast and choriocarcinoma cells. Exp Cell Res 1998;244(1):249–58.
[33] Nakanishi T, Kohroki J, Suzuki S, Ishizaki J, Hiromori Y, Takasuga S, et al. Trialkyltin compounds enhance human CG secretion and aromatase activity in human placental choriocarcinoma cells. J Clin Endocrinol Metab 2002;87(6):2830–7.
[34] Burleigh DW, Kendziorski CM, Choi YJ, Grindle KM, Grendell RL, Magness RR, et al. Microarray analysis of BeWo and JEG3 trophoblast cell lines: identification of differentially expressed transcripts. Placenta 2007;28(5–6):383–9.
[35] Imir AG, Lin Z, Yin P, Deb S, Yilmaz B, Cetin M, et al. Aromatase expression in uterine leiomyomata is regulated primarily by proximal promoters I.3/II. J Clin Endocrinol Metab 2007;92(5):1979–82.
[36] Mendelson CR, Jiang B, Shelton JM, Richardson JA, Hinshelwood MM. Transcriptional regulation of aromatase in placenta and ovary. J Steroid Biochem Mol Biol 2005;95(1–5):25–33.
[37] Kamat A, Smith ME, Shelton JM, Richardson JA, Mendelson CR. Genomic regions that mediate placental cell-specific and developmental regulation of human CYP19 (aromatase) gene expression in transgenic mice. Endocrinology 2005;146(5):2481–8.
[38] Luo S, Yu H, Wu D, Peng C. Transforming growth factor-beta1 inhibits steroidogenesis in human trophoblast cells. Mol Hum Reprod 2002;8(4):318–25.
[39] Toda K, Yang LX, Shizuta Y. Transcriptional regulation of the human aromatase cytochrome P450 gene expression in human placental cells. J Steroid Biochem Mol Biol 1995;53(1–6):181–90.
[40] Wang J, Chen S. Induction of aromatase gene expression in human placental choriocarcinoma (JAR) cells by phorbol esters. Biochim Biophys Acta 1994;1218:48–54.
[41] Banes AK, Shaw SM, Tawfik A, Patel BP, Ogbi S, Fulton D, et al. Activation of the JAK/STAT pathway in vascular smooth muscle by serotonin. Am J Physiol Cell Physiol 2005;288(4):C805–12.
[42] Singh RK, Dai Y, Staudinger JL, Muma NA. Activation of the JAK-STAT pathway is necessary for desensitization of 5-HT_{2A} receptor-stimulated phospholipase

C signalling by olanzapine, clozapine and MDL 100907. Int J Neuropsychopharmacol 2009;12(5):651–65.
[43] Zhao Y, Nichols JE, Bulun SE, Mendelson CR, Simpson ER. Aromatase P450 gene expression in human adipose tissue. Role of a Jak/STAT pathway in regulation of the adipose-specific promoter. J Biol Chem 1995;270(27):16449–57.
[44] Bonnin A, Goeden N, Chen K, Wilson ML, King J, Shih JC, et al. A transient placental source of serotonin for the fetal forebrain. Nature 2011;472(7343):347–50.
[45] Kallen B. The safety of antidepressant drugs during pregnancy. Expert Opin Drug Saf 2007;6(4):357–70.
[46] Way CM. Safety of newer antidepressants in pregnancy. Pharmacotherapy 2007;27(4):546–52.
[47] Wisner KL, Sit DK, Hanusa BH, Moses-Kolko EL, Bogen DL, Hunker DF, et al. Major depression and antidepressant treatment: impact on pregnancy and neonatal outcomes. Am J Psychiatry 2009;166(5):557–66.
[48] Bolte AC, van Geijn HP, Dekker GA. Pathophysiology of preeclampsia and the role of serotonin. Eur J Obstet Gynecol Reprod Biol 2001;95(1):12–21.
[49] Viau M, Lafond J, Vaillancourt C. Expression of placental serotonin transporter and 5-HT 2A receptor in normal and gestational diabetes mellitus pregnancies. Reprod Biomed Online 2009;19(2):207–15.