0031-3998/04/5601-0040
PEDIATRIC RESEARCH
Vol. 56, No. 1, 2004
Printed in U.S.A.

Chronic Maternal Fluoxetine Infusion in Pregnant Sheep: Effects on the Maternal and Fetal Hypothalamic-Pituitary-Adrenal Axes

JANNA L. MORRISON, K. WAYNE RIGGS, CALY CHIEN, NANCY GRUBER, I. CAROLINE MCMILLEN, DAN W. RURAK

Department of Obstetrics and Gynaecology, British Columbia Research Institute for Children's & Women's Health, Vancouver, British Columbia, Canada V5Z 4H4 [J.L.M., N.G., D.W.R.], Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3 [K.W.R., C.C.], and Department of Physiology, Adelaide University, Adelaide, South Australia, Australia 5005 [I.C.M.]

ABSTRACT

Depression during pregnancy is frequently treated with the selective serotonin reuptake inhibitor, fluoxetine (FX). FX increases serotonergic neurotransmission and serotonin plays a role in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. We have therefore investigated the effect of chronic administration of FX to the pregnant ewe on the maternal and fetal HPA axes. Nineteen late-gestation sheep were surgically prepared for chronic study of the fetus. FX ($n = 7$, 98.5 μg/kg/d) or sterile water (control, $n = 8$) was administered to the ewe for 8 d by constant rate i.v. infusion with an initial FX bolus dose of 70 mg. Maternal and fetal plasma ACTH and cortisol concentrations were determined at 0700 h each day. Maternal plasma ACTH concentrations fell on infusion d 2, but no changes were observed in maternal plasma cortisol concentrations. Fetal plasma ACTH concentrations increased on infusion d 7, and fetal plasma cortisol concentrations increased on infusion d 6, 7, and 8 in the FX group. In addition, the regression coefficient for the relationship between fetal ACTH and cortisol levels was significantly greater in the FX group compared with the control group. Thus, maternal FX treatment increased fetal plasma cortisol concentration. These results are of particular interest in the context that exposure of the fetus to excess glucocorticoids at critical windows during development has been shown to increase the risk of poor health outcomes in later life. **(*Pediatr Res* 56: 40–46, 2004)**

Abbreviations

SSRI, selective serotonin reuptake inhibitor
FX, fluoxetine
HPA, hypothalamic-pituitary-adrenal
ACTH, adrenocorticotrophin
NFX, norfluoxetine
CRH, corticotrophin-releasing hormone
AVP, arginine vasopressin

Clinical depression occurs in 5–15% of pregnant women (1). Treatment of depression during pregnancy is important to fetal outcome by preventing poor maternal self-care and nutrition, disturbed sleep, lack of prenatal care, increased exposure to alcohol and drugs, and a higher risk of suicide by the mother (1). Depression at 28 wk gestation is related to an increase in negative pregnancy outcome, including an increased risk of low-birth-weight newborns, preterm delivery, and small-for-gestational-age newborns (2). FX is a SSRI that increases serotonergic neurotransmission and is prescribed clinically for the treatment of depression, obsessive-compulsive disorder, and bulimia (3). It and other SSRIs have become increasingly used for the treatment of depression in pregnancy because of their effectiveness and lower incidence of maternal side effects and wider safety margin compared with tricyclic antidepressants and monoamine oxidase inhibitors (4, 5). However, there have been several reports that use of these drugs during pregnancy can increase the incidence of adverse pregnancy outcomes, including preterm delivery, fetal growth restriction, and poor neonatal adaptation (5–8). There is also evidence for postnatal consequences of prenatal SSRI exposure, including reduced weight gain, slight delays in psychomotor development and motor movement control, and our own findings of reduced facial and heart rate responses to painful stimuli (9–11).

Received July 10, 2003; accepted November 11, 2003.
Correspondence: Janna Morrison, Ph.D., Medical School South, Rm. S515, Discipline of Physiology, School of Molecular and Biomedical Sciences, Adelaide University, Adelaide, SA, Australia, 5005; e-mail: janna.morrison@adelaide.edu.au
Supported by a grant from the Canadian Institutes of Health Research (CIHR). D.W.R. is the recipient of an investigatorship award from the British Columbia Research Institute for Children's & Women's Health. J.L.M. is the recipient of a CIHR Studentship. C.C. is the recipient of a PMAC/HRF-CIHR studentship. Novopharm Ltd., Toronto, ON, Canada, generously donated the fluoxetine hydrochloride used in these studies.

DOI: 10.1203/01.PDR.0000128981.38670.28

FX and other SSRIs increase extracellular serotonin (5-HT) levels acutely and serotonergic neurotransmission chronically, and these actions could underlie the reported adverse effects of these agents on pregnancy outcome and postnatal development (7, 10, 12). Serotonin plays a major role in the regulation of the HPA axis and adrenocortical secretion of cortisol (13). The actions of serotonin, released by neurons originating in the midbrain raphe nucleus, are mediated by 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors in the paraventricular nucleus of the hypothalamus (14–17).

Basal ACTH and cortisol plasma concentrations did not change in humans receiving 20 mg FX for 20 d (18). Most studies of chronic FX treatment, however, have focused on the neuroendocrine responses to a challenge of the serotonergic system with either 5-hydroxytryptophan (5-HTP), the precursor to 5-HT, or a 5-HT_{1A} agonist. Compared with controls, an attenuated ACTH and cortisol/corticosterone response to 5-HT_{1A} activation occurred after chronic FX treatment in humans (18, 19) and rats (20–22). Rats treated with 10 mg/kg FX for 21 d exhibited attenuated ACTH and cortisol responses to a 5-HT_{1A} agonist (8-OH-DPAT) (21). The FX-elicited blunting of ACTH and cortisol responses to 5-HT_{1A} receptor stimulation appears to involve desensitization of postsynaptic 5-HT_{1A} receptors (19), whereas the antidepressant actions of FX involve desensitization of presynaptic 5-HT_{1A} autoreceptors (3). Taken together, these studies illustrate that our current knowledge of the effects of FX on the endocrine system without serotoninergic challenge is incomplete. Furthermore, no studies have investigated the impact of maternal FX treatment on the fetal endocrine system.

Placental transfer of FX has been demonstrated in the rat (23), human (24), and sheep (25). In adults, FX is metabolized to norfluoxetine (NFX), which also has antidepressant activity; however, in pregnant sheep, NFX has not been detected in the fetus after fetal drug administration, suggesting that FX is not metabolized in the fetus (24, 26). FX may, however, have similar effects in the fetus as in the adult on serotonin neurotransmission and HPA axis function. However, only one study has investigated the interaction between serotonin and the fetal HPA axis. Intravenous infusion of 5-HTP to the sheep fetus at 110 and 130 d gestation increased serotonin in hypothalamic tissue (27). Thus, our hypothesis is that maternal FX treatment in sheep will increase fetal HPA axis function as measured by an increase in fetal plasma ACTH and cortisol concentration.

MATERIALS AND METHODS

Animals and surgical preparation. Nineteen time-bred Dorset/Suffolk cross, pregnant sheep were surgically prepared between 118 and 122 d gestation (term, 147 d). All experimental protocols and procedures performed on the sheep conformed to the guidelines of the Canadian Council on Animal Care and were approved by The University of British Columbia Animal Care Committee. Surgical procedures have been described in detail previously (28). Briefly, anesthesia was induced with 1 g pentothal and maintained with 1.5% isoflurane after intubation. A midline incision in the maternal abdomen allowed access to the uterus to expose the fetus for implantation of polyvinyl catheters (Tygon, Akron, OH, U.S.A.) in the trachea, femoral arteries, lateral tarsal vein, and amniotic cavity. As previously reported (12, 29), animals were instrumented for measurement of fetal behavioral state and uterine artery blood flow.

Experimental protocol. Ewes were housed in a 12:12 light/dark cycle (lights on at 0600 h) with grain and hay once daily between 0830 and 0900 h and additional hay between 1530 and 1600 h. Ewes recovered for 3–4 d after surgery before experiments began. A preinfusion day preceded an 8-d infusion of either sterile water ($n = 8$) or FX ($n = 7$). The control group consisted of six female and two male fetuses, whereas the FX group consisted of three female and four male fetuses. There were one singleton, six twins, and one set of triplets in the control group and three singletons and four twins in the FX group. At 0700 h on infusion d 1, a 10-mL bolus injection of either 70 mg FX or sterile water was given over 2 min into the maternal femoral vein catheter followed by continuous infusion of 98.5 μg/kg/d FX or an equivalent volume of sterile water for 8 d. The loading dose and infusion rate were calculated based on volume of distribution and systemic clearance data obtained from FX i.v. bolus pharmacokinetic studies in pregnant sheep previously performed in our laboratory (25). At 0700 h each day, maternal and fetal blood samples were collected for analysis of blood gases (1 mL) and plasma concentration of FX, cortisol, and ACTH (1.5 mL) and stored at −20°C for 2 wk and then at −80°C. To limit total blood volume removed, not all hormones were measured in all animals at all time points. All fetuses delivered spontaneously (12).

Blood gas analysis. Blood samples were analysed for pH, P_{CO_2} and P_{O_2} with an IL 1306 pH/blood gas analyser (Allied Instrumentation Laboratory, Milan, Italy) and temperature corrected to 39.5°C for fetal samples and 39°C for maternal samples. Hb and oxygen saturation were measured with an OSM-2 Hemoximeter (Radiometer, Copenhagen, Denmark).

Fluoxetine analysis. A rapid, sensitive, and selective chiral assay for FX and NFX enantiomers using gas chromatography mass spectrometry with selective ion monitoring developed in our laboratory was used for plasma FX analysis as previously described (25). Samples were analyzed for plasma FX and NFX concentrations only if maternal and fetal blood samples were collected at all time points in the protocols from 0700 h on the preinfusion day to 72 h postinfusion ($n = 6$).

ACTH hormone RIA. Plasma concentrations of immunoreactive ACTH were determined using an ^{125}I RIA kit (ICN Biomedicals, Seven Hills, NSW, Australia). The intra-assay coefficient of variation was <10% and the interassay coefficient of variation was 14.6%. The rabbit antihuman $ACTH^{1\rightarrow39}$ had a cross reactivity of <0.01% with α-melanocyte-stimulating hormone, β-endorphin, α-lipotropin, and β-lipotropin. The sensitivity of the assay was 7 pg/mL (30).

Cortisol RIA. Total cortisol plasma concentration determination was performed using an ^{125}I RIA kit (Orion Diagnostica, Espo, Finland). The average efficiency of recovery of ^{125}I cortisol using dichloromethane extraction was 90% (31). The sensitivity of the assay was 0.39 nmol/L. The rabbit anticortisol antibody cross-reacted <1% with cortisone and 17-hy-

Table 1. *Maternal and fetal plasma FX concentrations (*n = *6) at 0700 h daily*

	Maternal FX (ng/mL)	Fetal FX (ng/mL)
Preinf	0.0 ± 0.0	0.0 ± 0.0
Inf 1	0.0 ± 0.0	0.0 ± 0.0
Inf 2	84.0 ± 14.4	36.2 ± 8.8
Inf 3	112.8 ± 21.4	49.2 ± 10.2
Inf 4	128.6 ± 30.8	67.8 ± 17.4
Inf 5	147.3 ± 30.0	68.1 ± 11.9
Inf 6	159.7 ± 34.3	75.3 ± 13.1
Inf 7	177.8 ± 32.4	81.6 ± 14.7
Inf 8	199.2 ± 41.2	80.0 ± 16.3

Data given as mean ± SEM. Preinf, preinfusion day; Inf, infusion day.

droxyprogesterone and <0.01% with aldosterone, pregnenolone, estradiol, and progesterone. The inter- and intra-assay coefficients of variation were <10%.

Statistical analysis. Results are presented as means ± SEM. Blood gas, cardiovascular, and hormone data were analysed using repeated measures ANOVA followed by post hoc Fishers LSD test to determine the difference between the control and FX groups at each time point ($p < 0.05$) (denoted by [a] when $p < 0.05$) using NCSS Statistical Software (Kaysville, UT. U.S.A.). In addition, the effect of time on changes in measurements from preinfusion day (denoted by [b] in the control group and [c] in the FX group) was determined using repeated measures ANOVA followed by post hoc Fishers *t* tests ($p < 0.05$). Note that the preinfusion and infusion d 1 values are pre-FX exposure. Regression analysis of the relationship between fetal ACTH and cortisol concentrations was accomplished using the least squares method. Animals were excluded from analysis if the fetal cortisol plasma concentration was >15 nmol/L on the preinfusion day, as this was an indication that a premature prepartum increase in cortisol may have been present in these animals. One fetus in the FX-treated group died just before the onset of labor. Fetal blood gas and behavioral parameters were in the normal ranges until about 12 h before death. Accordingly, data from this animal were used only up to infusion d 5. Thus, cortisol and ACTH data were analyzed from eight control and seven FX-treated animals.

RESULTS

Fetal outcome. On infusion d 8, mean gestational age was 135.4 ± 0.6 d in the control group and 135.6 ± 0.7 d in the FX group and the number of days before delivery occurred after the end of the infusion period was 7.1 ± 1.6 in the control group and 6.3 ± 1.3 in the FX group. Birth weight was 3072.8 ± 299 g in the control group and 3645 ± 252 in the FX group. There was no significant difference in gestational age, number of days before delivery, or birth weight between the two groups. In the control group, seven of the fetuses were born alive and there were one intrauterine and two intrapartum deaths. In the FX group, four of the fetuses were born alive, but there was one neonatal death. There were also two intrapartum deaths and one death just before the onset of labor.

Maternal and fetal plasma fluoxetine concentration. On infusion d 1, FX levels in both the ewe and fetus peaked 5 min

Table 2. *Fetal blood gas status of animals at 0700 h daily*

	Preinf	Inf 1	Inf 2	Inf 3	Inf 4	Inf 5	Inf 6	Inf 7	Inf 8
pH									
Con (n = 8)	7.345 ± 0.011	−0.009 ± 0.006	−0.001 ± 0.013	−0.008 ± 0.012	−0.018 ± 0.010	−0.011 ± 0.013	−0.022 ± 0.020	−0.017 ± 0.020	−0.011 ± 0.017
FX (n = 7)	7.344 ± 0.007	−0.007 ± 0.007	−0.016 ± 0.008	−0.016 ± 0.008	−0.017 ± 0.002[c]	−0.009 ± 0.006	−0.029 ± 0.007[c]	−0.026 ± 0.005[c]	−0.012 ± 0.012
P_{CO_2} (mm Hg)									
Con (n = 8)	48.3 ± 0.9	0.7 ± 1.0	0.9 ± 1.1	0.7 ± 1.0	1.3 ± 1.1	1.5 ± 1.1	1.6 ± 1.0	1.6 ± 1.5	1.1 ± 1.3
FX (n = 7)	49.3 ± 1.3	0.9 ± 1.3	3.3 ± 1.4	0.9 ± 1.5	1.6 ± 1.8	0.5 ± 1.3	0.5 ± 1.1	1.6 ± 1.6	1.9 ± 1.2
P_{O_2} (mm Hg)									
Con (n = 8)	22.5 ± 1.5	−0.2 ± 0.6	−1.0 ± 0.7	−1.4 ± 1.0	−0.6 ± 1.3	−1.7 ± 1.3	−0.8 ± 0.9	0.0 ± 1.5	−0.4 ± 0.8
FX (n = 7)	23.0 ± 1.1	0.1 ± 0.7	−3.0 ± 1.3[c]	−0.6 ± 0.9	−1.6 ± 1.6	−2.1 ± 0.7[c]	−3.3 ± 0.9	−2.4 ± 0.6[c]	−1.3 ± 0.9
Hemoglobin (g%)									
Con (n = 8)	10.9 ± 0.8	0.0 ± 0.3	−0.6 ± 0.4	−1.0 ± 0.4[b]	−0.8 ± 0.4[b]	−1.0 ± 0.3[b]	−1.1 ± 0.3[b]	−0.9 ± 0.4[b]	−1.0 ± 0.4[b]
FX (n = 7)	10.7 ± 0.9	0.0 ± 0.4	0.4 ± 0.3	0.0 ± 0.4	0.2 ± 0.4	−0.3 ± 0.4	−0.3 ± 0.3	−0.2 ± 0.5	0.1 ± 0.4
Oxygen saturation (%)									
Con (n = 8)	55.7 ± 4.1	−2.6 ± 2.0	−3.9 ± 2.3	−5.0 ± 2.5	−6.1 ± 3.3[b]	−7.5 ± 3.4[b]	−7.1 ± 1.6[b]	−6.8 ± 3.5[b]	−9.8 ± 2.6[b]
FX (n = 7)	59.1 ± 3.1	0.4 ± 1.0	−12.8 ± 4.3[c]	−4.0 ± 2.0	−8.4 ± 2.1[c]	−6.7 ± 2.7[c]	−11.0 ± 3.8[c]	−11.8 ± 2.1[c]	−9.6 ± 2.6[c]

Absolute value is shown for the preinfusion day, with change from preinfusion day shown thereafter. Data given as mean ± SEM. Significant difference ($p < 0.05$) from preinfusion day where [b] is the control group and [c] is the FX group. Preinf, preinfusion day; Inf, infusion day; Con, control.

Table 3. *Daily average maternal and fetal arterial pressure*

	Preinf	Inf 1	Inf 2	Inf 3	Inf 4	Inf 5	Inf 6	Inf 7	Inf 8
Maternal	(mm Hg)								
Con ($n = 5$)	80.3 ± 6.9	75.1 ± 8.2	76.3 ± 7.8	72.8 ± 7.9	71.4 ± 6.8	73.5 ± 7.4	73.1 ± 6.1	76.8 ± 6.8	76.4 ± 4.2
FX ($n = 6$)	88.4 ± 6.4	87.4 ± 6.3	86.1 ± 5.7	85.6 ± 5.3	85.3 ± 5.6	87.5 ± 6.0	89.0 ± 7.3	88.4 ± 6.8	89.5 ± 9.2
Fetal	(mm Hg)								
Con ($n = 5$)	45.3 ± 2.1	44.5 ± 1.8	44.5 ± 1.4	45.0 ± 1.8	44.2 ± 1.4	44.2 ± 2.5	45.6 ± 2.0	47.6 ± 2.7	44.6 ± 3.4
FX ($n = 6$)	45.1 ± 1.7	46.8 ± 0.9	47.1 ± 1.5	46.1 ± 1.4	45.8 ± 1.0	47.3 ± 0.7	46.2 ± 1.9	46.2 ± 0.9	47.1 ± 1.2

Data given as mean ± SEM. No significant differences were observed between the control and FX group or across the experimental period in either group. Preinf, preinfusion day; Inf, infusion day; Con, control.

after the bolus dose and subsequently decreased during the first 6 h (Table 1). Thereafter, maternal and fetal FX concentrations progressively increased throughout the 8-d infusion, peaking at 199.2 ± 41.2 ng/mL in the ewe and at 80.0 ± 16.3 ng/mL in the fetus on infusion d 8.

Fetal blood gas status. In the FX group, fetal arterial Po_2 was significantly below the preinfusion day value (23.0 ± 1.1 mm Hg) on infusion d 2, 5, and 7 ($p < 0.05$), as indicated in Table 2. Fetal oxygen saturation was also lower ($p < 0.05$) than the preinfusion day value (59.1 ± 3.1%) on infusion d 2, 4, 5, 6, 7, and 8 in the FX group. A decrease in oxygen saturation ($p < 0.05$) was also observed in the control group on infusion d 4, 5, 6, 7, and 8 compared with the preinfusion day (55.7 ± 4.1%), with the maximum drop of 9.8 ± 2.6% on infusion d 8. Fetal pH was also lower ($p < 0.05$) on infusion d 4, 6, and 7 than on the preinfusion day in the FX group. There were no changes in Pco_2 in either the control or FX groups; however, Hb concentrations decreased significantly in the control group on infusion d 3, 4, 5, 6, 7, and 8. No changes in the daily average arterial pressure were observed in the ewes or fetuses within or between the control ($n = 5$) and FX ($n = 6$) groups throughout the experimental period (Table 3).

Plasma ACTH and cortisol concentrations. There was no significant difference in maternal or fetal ACTH between the control and FX groups during the preinfusion period (Fig. 1, *A* and *B*). There was, however, a significant ($p < 0.05$) fall from the preinfusion day in maternal plasma ACTH concentrations in the FX group on infusion d 2 and 3 (Fig. 1*A*), the maximum fall occurring on infusion d 2 (−38.0 ± 11.9 pg/mL). In contrast, there were no significant changes in maternal ACTH throughout the period of the infusion in the control group. Maternal cortisol concentrations did not change throughout the infusion period in either the FX or control group (Fig. 2*A*). When ACTH and cortisol concentrations early in the protocol (average concentration on the preinfusion day, infusion d 1 and 2) and late in the protocol (average concentration on infusion d 5–8) were pooled, there was a significant increase in maternal cortisol on infusion d 5–8 in the FX group (46.8 ± 11.6 nmol/L) compared with preinfusion to infusion d 2 (24.7 ± 3.4 nmol/L) in the absence of any change in maternal ACTH.

In the fetus, there was a significant ($p < 0.05$) increase in plasma ACTH concentrations from 37.0 ± 7.9 pg/mL on preinfusion day to 75.7 ± 20.1 on infusion d 7 in the FX group (Fig. 1*B*). There was a significant increase in the fetal plasma cortisol concentrations on infusion d 6, 7, and 8 compared with the preinfusion day in both the control and FX groups (Fig. 2*B*). The maximum change in fetal cortisol occurred on infusion d 7 in both groups, with an increase of 27.0 ± 15.7 nmol/L in the control group and 58.5 ± 16.2 nmol/L in the FX group. The change in fetal cortisol concentration on infusion d 7 and 8 was significantly greater in the FX group than the control group ($p < 0.05$). In the FX-exposed fetuses, the increase in cortisol [from 34.3 ± 4.6 nmol/L (preinfusion to infusion d 2) to 87.4 ± 21.1 nmol/L (infusion d 6–8)] was significantly greater when compared with the control group (from 47.0 ± 5.1 nmol/L to 54.0 ± 10.2 nmol/L). Fetal cortisol concentrations increased in both the control (29.8 ± 6.6 nmol/L) and FX (53.2 ± 7.4 nmol/L) groups on infusion d 5–8 when compared with preinfusion to infusion d 2 (8.7 ± 1.2 nmol/L, control group; 6.8 ± 1.0 nmol/L, FX group), and this increase was significantly greater in the FX group ($p < 0.05$).

There was a significant relationship between plasma cortisol (Y) and ACTH (X) concentrations in both the FX and control

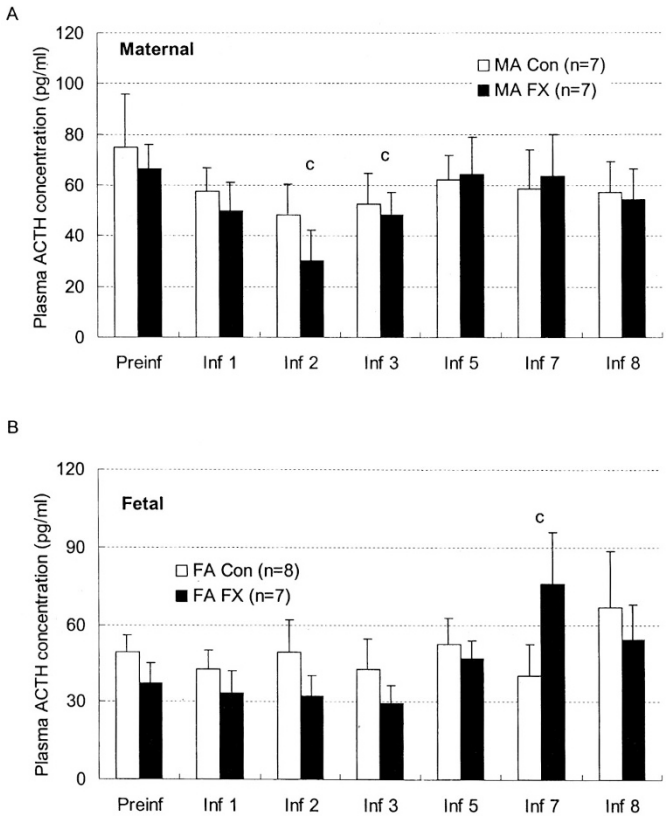

Figure 1. Maternal (*A*) and fetal (*B*) arterial plasma ACTH concentrations at 0700 h on the preinfusion day and 8 d of sterile water (Con) or FX infusion. [c]Significant difference ($p < 0.05$) from preinfusion day FX group. Preinf, preinfusion day; Inf, infusion day; MA, maternal arterial; FA, fetal arterial.

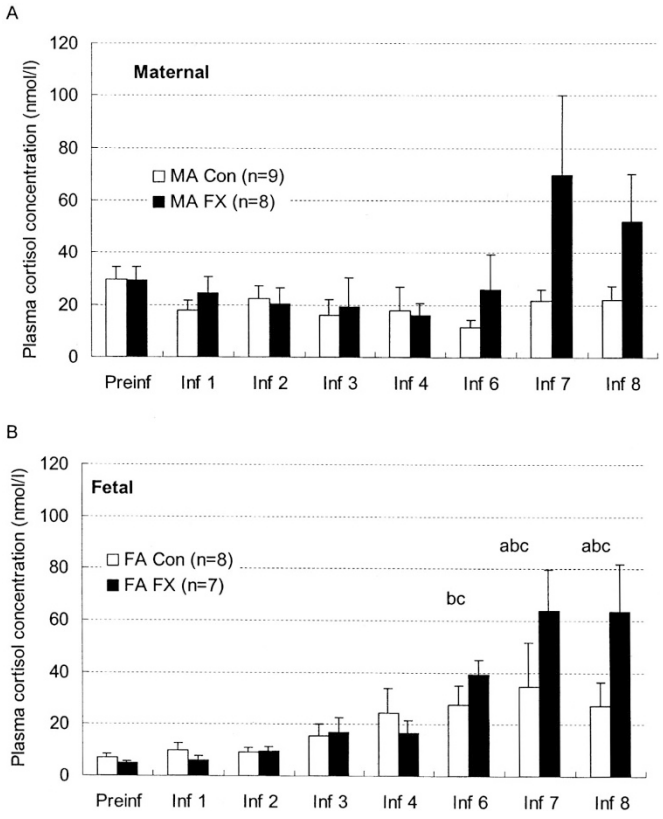

Figure 2. Maternal (*A*) and fetal (*B*) arterial plasma cortisol concentrations at 0700 h on the preinfusion day and 8 d of sterile water (*Con*) or FX infusion. [a]Significant difference ($p < 0.05$) between control and FX groups; significant difference ($p < 0.05$) from preinfusion day in [b]control and [c]FX group. Preinf, preinfusion day; Inf, infusion day; MA, maternal arterial; FA, fetal arterial.

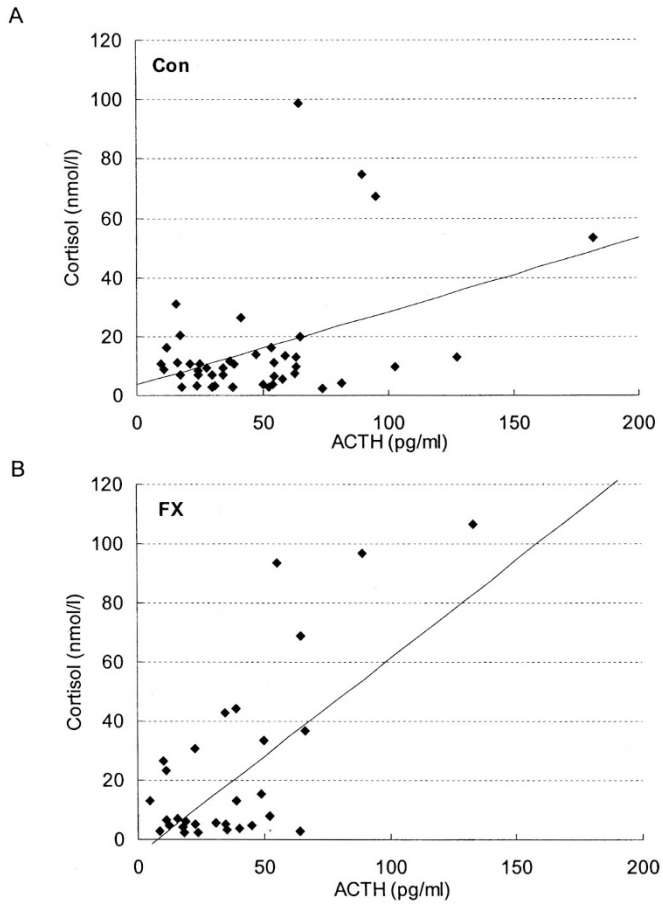

Figure 3. Relationship between fetal plasma ACTH and cortisol concentrations in control (*A*) and FX-exposed (*B*) fetuses. The regression equation for the control group is $Y = 0.249 \pm 0.085 * X + 3.59$ ($r = 0.1703$, $p = 0.005$, $n = 43$) and for the FX group is $Y = 0.662 \pm 0.144 * X - 5.03$ ($r^2 = 0.3896$, $p = 0.0006$, $n = 35$). Con, control group; FX, fluoxetine group.

groups, as shown in Figure 3. The slope of the relationship, however, was significantly greater ($p = 0.02$) in the FX group than the control group [FX: $Y = 0.662 \pm 0.144 * X - 5.03$ ($r^2 = 0.3896$, $p = 0.0006$, $n = 35$); control: $Y = 0.249 \pm 0.085 * X + 3.59$ ($r = 0.1703$, $p = 0.005$, $n = 43$)].

DISCUSSION

Our results indicate that an 8-d maternal i.v. infusion of FX has little effect on the maternal pituitary-adrenal axis while increasing the magnitude of the normal prepartum rise in fetal plasma cortisol concentration. Although there was a small fall in maternal plasma ACTH on infusion d 2 and 3 in the FX, but not the control group, overall there was no significant effect of FX treatment on maternal plasma cortisol concentration. In contrast, there was a greater rise in fetal plasma ACTH and cortisol in the FX group when compared with the control fetuses.

It has been reported that fetal ACTH plasma levels increase progressively between 110 and 140 d gestation, whereas fetal cortisol levels remain low between 110–120 d and increase between 125–140 d gestation (32). McMillen *et al.* (33) also found that fetal plasma ACTH concentrations double between 120 and 136 d and 140 and 143 d gestation. In the current study, fetal plasma ACTH concentrations did not increase in the control group but doubled in the FX group on infusion d 7. One possibility is that FX treatment induced a premature increase in fetal ACTH at an earlier gestational age (mean 134.5 ± 1.0 d). Alternatively, FX may have acted within the fetal HPA axis either to increase the secretion of the hypothalamic secretagogues, CRH or AVP, or to enhance the sensitivity of the corticotrophs within the fetal pituitary to the actions of CRH or AVP.

Previous studies have reported an increase in plasma cortisol levels in fetal lambs in late gestation, beginning about 9 d before delivery (34, 35). In the current study, this corresponds to infusion d 5 and 4 in control and FX-exposed fetuses, respectively. Thus, the increase in plasma cortisol concentrations between infusion d 4 and 8 in both the control and FX groups is likely to represent the start of the prepartum rise in cortisol. In the present study, we found that the increase in fetal cortisol was greater in the FX-treated group on infusion d 5–8, and that plasma cortisol concentrations appeared to be higher in this group at any given ACTH concentration. This suggests that the fetal adrenal sensitivity to ACTH may have increased in the FX group (36). It is possible that FX treatment resulted in a stimulation of the secretion of proopiomelanocortin (POMC) products other than ACTH from the fetal pituitary. It has been shown that N-POMC 1–77 has a stimulatory action on fetal adrenal growth and steroidogenic enzyme expression

during late gestation (34). An alternative possibility is that FX acted in the fetal CNS to increase stimulation of the fetal adrenal by the cholinergic preganglionic splanchnic nerve. It has been demonstrated that the splanchnic nerve plays a role in the modulation of the adrenal responsiveness to ACTH stimulation (37).

Acute hypoxemia and acidemia are potent stimuli for ACTH and cortisol release in the fetus (31, 38–44). Although there were small decreases in fetal arterial Po_2 in the FX groups during the infusion period, the fetal arterial Po_2 values did not fall below levels associated with a pituitary-adrenal response to fetal hypoxemia in either acute or chronic studies (39, 40). It is therefore unlikely that the changes in fetal blood gas status contributed to the fetal pituitary-adrenal response to maternal FX infusion.

It has previously been shown that cortisol plays a role in the regulation of fetal blood pressure. An intrafetal infusion of cortisol for 24 h during the period between 103 and 120 d gestation resulted in an increase in fetal plasma cortisol concentrations to levels similar to those observed close to term and a concomitant increase in blood pressure (45). Although changes in fetal blood pressure and heart rate were observed in the first 24 h after FX infusion (12), these changes are not associated with changes in fetal cortisol concentrations and there were no changes in either maternal or fetal blood pressure and heart rate during the 8-d FX infusion period. Although infusion of cortisol between 103 and 120 d gestation resulted in an increase in fetal blood pressure, infusion of cortisol for a 24-h period between 130 and 137 d did not result in any change in fetal arterial blood pressure, and it is likely that further elevations of fetal cortisol above those levels normally present during the prepartum period will not result in further elevations in fetal blood pressure (45).

Maternal FX plasma concentrations rose on each day of the experiment, but were within the human therapeutic range of 35–415 ng/mL (12, 46). Changes in fetal plasma ACTH and cortisol concentrations were not observed during the first 5 d of FX treatment despite high plasma FX concentrations. This may be related to serotonin receptor function. One of the effects of the rise in extracellular serotonin elicited by FX and other SSRIs is to down-regulate the $5\text{-}HT_{1A}$ autoreceptors that inhibit the firing of serotonergic neurons in the raphe nucleus. However, in adults, this appears to require 2–3 wk of FX treatment (47). In the fetus, the development of the brain serotonin system begins early in gestation, with serotonin immunoreactive perikarya present in the 110 d gestation sheep fetus with well-developed neuritic processes (48). During development, serotonin receptors may be more susceptible to down-regulation than in the adult. In rats, increased serotonin concentrations during pregnancy induced through a tryptophan-enriched diet or through 5-methoxytryptophan treatment delays serotonin axon outgrowth and/or decreases collateral sprouting, synapse formation, and induces receptor down-regulation (49, 50). Pharmacodynamic modelling was performed to determine whether there was a relationship between maternal or fetal FX concentrations and ACTH or cortisol concentrations but no correlations were found (data not shown).

Our data show that maternal FX administration increases fetal plasma cortisol concentration during late gestation. Previously we have reported that maternal FX infusion reduces the incidence of low-voltage/rapid eye movement (LV/REM) behavioral state in the fetal lamb, and that this effect persists for the entire 8-d infusion period (29). Thus, FX exposure alters the level of activation of both the HPA axis and fetal behavior (13) and these alterations could underlie some of the postnatal consequences observed in human infants exposed prenatally to SSRIs (7, 9–11). What is not yet clear is whether the FX-elicited alterations in the fetal HPA axis persist beyond the period of drug exposure and into the postnatal period. Study of postnatal HPA function after *in utero* SSRI exposure is required to substantiate this possibility. It has been shown in experimental animal studies that exposure to synthetic glucocorticoids during late gestation results in a subsequent increase in blood pressure, in the hepatic production of glucose and in basal cortisol concentrations in adult life (51). Thus exposure to excess glucocorticoids *in utero* as a consequence of exposure of the fetus to therapeutic agents such as the synthetic glucocorticoids used in the treatment of threatened preterm labor (52) or the SSRIs may have consequences for fetal and postnatal health.

Acknowledgment. The authors thank Anne Jurisevic for her expert research assistance.

REFERENCES

1. Weissman MM, Olfson M 1995 Depression in women: implications for health care research. Science 269:799–801
2. Steer RA, Scholl TO, Hediger ML, Fischer RL 1992 Self-reported depression and negative pregnancy outcomes. J Clin Epidemiol 45:1093–1099
3. Stahl SM 1998 Basic psychopharmacology of antidepressants, part 1: antidepressants have seven distinct mechanisms of action. J Clin Psychiatry 59:5–14
4. Nonacs R, Cohen LS 2002 Depression during pregnancy: diagnosis and treatment options. J Clin Psychiatry 63:24–30
5. Simon GE, Cunningham ML, Davis RL 2002 Outcomes of prenatal antidepressant exposure. Am J Psychiatry 159:2055–2061
6. Chambers CD, Johnson KA, Dick LM, Felix RJ, Jones KL 1996 Birth outcomes in pregnant women taking fluoxetine. N Engl J Med 335:1010–1015
7. Laine K, Heikkinen T, Ekblad U, Kero P 2003 Effects of exposure to selective serotonin reuptake inhibitors during pregnancy on serotonergic symptoms in newborns and cord blood monoamine and prolactin concentrations. Arch Gen Psychiatry 60:720–726
8. Hendrick V, Smith LM, Suri R, Hwang S, Haynes D, Altshuler L 2003 Birth outcomes after prenatal exposure to antidepressant medication. Am J Obstet Gynecol 188:812–815
9. Chambers CD, Anderson PO, Thomas RG, Dick LM, Felix RJ, Johnson KA, Jones KL 1999 Weight gain in infants breastfed by mothers who take fluoxetine. Pediatrics 104:E61
10. Casper RC, Fleisher BE, Lee-Ancajas JC, Gilles A, Gaylor E, DeBattista A, Hoyme HE 2003 Follow-up of children of depressed mothers exposed or not exposed to antidepressant drugs during pregnancy. J Pediatr 142:402–408
11. Oberlander TF, Eckstein Grunau R, Fitzgerald C, Ellwood AL, Misri S, Rurak D, Riggs KW 2002 Prolonged prenatal psychotropic medication exposure alters neonatal acute pain response. Pediatr Res 51:443–453
12. Morrison JL, Chien C, Riggs KW, Gruber N, Rurak D 2002 Effect of maternal fluoxetine administration on uterine blood flow, fetal blood gas status, and growth. Pediatr Res 51:433–442
13. Delarue C, Contesse V, Lenglet S, Sicard F, Perraudin V, Lefebvre H, Kodjo M, Leboulenger F, Yon L, Gallo-Payer N, Vaudry H 2001 Role of neurotransmitters and neuropeptides in the regulation of the adrenal cortex. Rev Endocr Metab Disord 2:253–267
14. Carrasco GA, Van de Kar LD 2003 Neuroendocrine pharmacology of stress. Eur J Pharmacol 463:235–272
15. Fuller RW 1996 Serotonin receptors involved in regulation of pituitary-adrenocortical function in rats. Behav Brain Res 73:215–219
16. Kageyama K, Tozawa F, Horiba N, Watanobe H, Suda T 1998 Serotonin stimulates corticotropin-releasing factor gene expression in the hypothalamic paraventricular nucleus of conscious rats. Neurosci Lett 243:17–20

17. Bagdy G 1996 Role of the hypothalamic paraventricular nucleus in 5-HT1A, 5-HT2A and 5-HT2C receptor-mediated oxytocin, prolactin and ACTH/corticosterone responses. Behav Brain Res 73:277–280
18. Berlin I, Warot D, Legout V, Guillemant S, Schollnhammer G, Puech AJ 1998 Blunted 5-HT1A-receptor agonist-induced corticotropin and cortisol responses after long-term ipsapirone and fluoxetine administration to healthy subjects. Clin Pharmacol Ther 63:428–436
19. Lerer B, Gelfin Y, Gorfine M, Allolio B, Lesch KP, Newman ME 1999 5-HT1A receptor function in normal subjects on clinical doses of fluoxetine: blunted temperature and hormone responses to ipsapirone challenge. Neuropsychopharmacology 20:628–639
20. Raap DK, Evans S, Garcia F, Li Q, Muma NA, Wolf WA, Battaglia G, Van De Kar LD 1999 Daily injections of fluoxetine induce dose-dependent desensitization of hypothalamic 5-HT1A receptors: reductions in neuroendocrine responses to 8-OH-DPAT and in levels of Gz and Gi proteins. J Pharmacol Exp Ther 288:98–106
21. Li Q, Levy AD, Cabrera TM, Brownfield MS, Battaglia G, Van de Kar LD 1993 Long-term fluoxetine, but not desipramine, inhibits the ACTH and oxytocin responses to the 5-HT1A agonist, 8-OH-DPAT, in male rats. Brain Res 630:148–156
22. Li Q, Brownfield MS, Levy AD, Battaglia G, Cabrera TM, Van de Kar LD 1994 Attenuation of hormone responses to the 5-HT1A agonist ipsapirone by long-term treatment with fluoxetine, but not desipramine, in male rats. Biol Psychiatry 36:300–308
23. Pohland RC, Byrd TK, Hamilton M, Koons JR 1989 Placental transfer and fetal distribution of fluoxetine in the rat. Toxicol Appl Pharmacol 98:198–205
24. Kim J 2000 Pharmacokinetics and pharmacodynamics of the selective serotonin reuptakes inhibitors, fluoxetine and paroxetine, during pregnancy and the nursing period. Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver
25. Kim J, Riggs KW, Rurak DW 2004 Stereoselective pharmacokinetics of fluoxetine and norfluoxetine enantiomers in pregnant sheep. Drug Metab Disp 32:212–221
26. Chien C 2003 Chronic exposure and pharmacokinetics of fluoxetine and norfluoxetine enantiomers in the pregnant ewe and fetus. Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver
27. Richards GE, Kendal JZ 1987 Effect of intravenous 5-hydroxytryptophan on hypothalamic concentration of norepinephrine, dopamine, serotonin and hydroxyindole acetic acid in the fetal lamb. Life Sci 40:2001–2005
28. Rurak DW, Yoo SD, Kwan E, Taylor SM, Riggs KW, Axelson JE 1988 Effects of diphenhydramine in the fetal lamb after maternal or fetal administration. J Pharmacol Exp Ther 247:271–278
29. Morrison JL, Chien C, Gruber N, Rurak D, Riggs W 2001 Fetal behavioural state changes following maternal fluoxetine infusion in sheep. Brain Res Dev Brain Res 131:47–56
30. Adams MB, Ross JT, Butler TG, McMillen IC 1999 Glucocorticoids decrease phenylethanolamine N-methyltransferase mRNA expression in the immature foetal sheep adrenal. J Neuroendocrinol 11:569–575
31. Bocking AD, McMillen IC, Harding R, Thorburn GD 1986 Effect of reduced uterine blood flow on fetal and maternal cortisol. J Dev Physiol 8:237–245
32. Norman LJ, Lye SJ, Wlodek ME, Challis JR 1985 Changes in pituitary responses to synthetic ovine corticotrophin releasing factor in fetal sheep. Can J Physiol Pharmacol 63:1398–1403
33. McMillen IC, Phillips ID, Ross JT, Robinson JS, Owens JA 1995 Chronic stress—the key to parturition? Reprod Fertil Dev 7:499–507
34. Kitts DD, Anderson GB, BonDurant RH, Stabenfeldt GH 1984 Temporal patterns of delta 4 C-21 steroids in coexisting, genetically dissimilar twin lamb fetuses throughout late gestation. Endocrinology 114:703–711
35. Magyar DM, Fridshal D, Elsner CW, Glatz T, Eliot J, Klein AH, Lowe KC, Buster JE, Nathanielsz PW 1980 Time-trend analysis of plasma cortisol concentrations in the fetal sheep in relation to parturition. Endocrinology 107:155–159
36. Brooks AN, Challis JR, Norman LJ 1987 Pituitary and adrenal responses to pulsatile ovine corticotropin-releasing factor administered to fetal sheep. Endocrinology 120:2383–2388
37. Myers DA, Robertshaw D, Nathanielsz PW 1990 Effect of bilateral splanchnic nerve section on adrenal function in the ovine fetus. Endocrinology 127:2328–2335
38. Boddy K, Dawes GS, Fisher R, Pinter S, Robinson JS 1974 Foetal respiratory movements, electrocortical and cardiovascular responses to hypoxaemia and hypercapnia in sheep. J Physiol 243:599–618
39. Towell ME, Figueroa J, Markowitz S, Elias B, Nathanielsz P 1987 The effect of mild hypoxemia maintained for twenty-four hours on maternal and fetal glucose, lactate, cortisol, and arginine vasopressin in pregnant sheep at 122 to 139 days' gestation. Am J Obstet Gynecol 157:1550–1557
40. Akagi K, Challis JR 1990 Threshold of hormonal and biophysical responses to acute hypoxemia in fetal sheep at different gestational ages. Can J Physiol Pharmacol 68:549–555
41. Challis JR, Richardson BS, Rurak D, Wlodek ME, Patrick JE 1986 Plasma adrenocorticotropic hormone and cortisol and adrenal blood flow during sustained hypoxemia in fetal sheep. Am J Obstet Gynecol 155:1332–1336
42. Hooper SB, Coulter CL, Deayton JM, Harding R, Thorburn GD 1990 Fetal endocrine responses to prolonged hypoxemia in sheep. Am J Physiol 259:R703–R708
43. Murotsuki J, Gagnon R, Matthews SG, Challis JR 1996 Effects of long-term hypoxemia on pituitary-adrenal function in fetal sheep. Am J Physiol 271:E678–E685
44. Gagnon R, Lamb T, Richardson B 1997 Cerebral circulatory responses of near-term ovine fetuses during sustained fetal placental embolization. Am J Physiol 273:H2001–H2008
45. Tangalakis K, Lumbers ER, Moritz KM, Towstoless MK, Wintour EM 1992 Effect of cortisol on blood pressure and vascular reactivity in the ovine fetus. Exp Physiol 77:709–717
46. Benfield P, Heel RC, Lewis SP 1986 Fluoxetine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in depressive illness. Drugs 32:481–508
47. Lanfumey L, Hamon M 2000 Central 5-HT(1A) receptors: regional distribution and functional characteristics. Nucl Med Biol 27:429–435
48. Tillet Y 1988 Early ontogeny of serotonin-immunoreactivity in the sheep brain. An immunohistochemical study. Anat Embryol (Berl) 178:429–440
49. Huether G, Thomke F, Adler L 1992 Administration of tryptophan-enriched diets to pregnant rats retards the development of the serotonergic system in their offspring. Brain Res Dev Brain Res 68:175–181
50. Whitaker-Azmitia PM, Lauder JM, Shemmer A, Azmitia EC 1987 Postnatal changes in serotonin receptors following prenatal alterations in serotonin levels: further evidence for functional fetal serotonin receptors. Brain Res 430:285–289
51. Levitt NS, Lindsay RS, Holmes MC, Seckl JR 1996 Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. Neuroendocrinology 64:412–418
52. Newnham JP, Moss TJ, Nitsos I, Sloboda DM 2002 Antenatal corticosteroids: the good, the bad and the unknown. Curr Opin Obstet Gynecol 14:607–612