Transient Loss of the Diurnal Rhythms of Fetal Movements, Heart Rate, and Its Variation after Maternal Betamethasone Administration

STEVEN V. KOENEN, EDUARD J.H. MULDER, LIA D. WIJNBERGER,
AND GERARD H.A. VISSER

Department of Perinatology and Gynecology, University Medical Center Utrecht, 3508 GA Utrecht, The Netherlands

ABSTRACT

Antenatal betamethasone administration to enhance fetal lung maturation is associated with transient reductions in fetal heart rate (FHR) variation, breathing, and body movements 2 d after the first dose (d 2). This study examines whether steroid administration affects the natural diurnal rhythms of fetal variables. Sixteen women at 27–32 wk of gestation received two doses of betamethasone 24 h apart. One-hour recordings of FHR, breathing, and body movements were made in the morning, afternoon, and evening of d 2, and again in the morning of d 3. Repeat recordings were obtained at 4–6 d later from 9/16 women. Maternal blood samples were obtained with each recording to determine ACTH and cortisol. No diurnal rhythm was present for FHR, FHR variation, breathing, and body movements on d 2. This resulted from suppression of the expected natural rise in body and breathing movements, and heart rate variation in the course of the day. Suppression of the diurnal rhythm of body movements depended on gestation ($R = -0.89$; $p < 0.01$). All

variables showed diurnal rhythms 4–6 d later. Maternal ACTH and cortisol diurnal rhythms were completely suppressed on d 2. Four to six days later, the normal diurnal pattern was resumed, although absolute levels of ACTH and cortisol were still suppressed. We conclude that maternal betamethasone administration transiently abolishes the fetal diurnal rhythms of heart rate and its variation, breathing, and body movements. (*Pediatr Res* 57: 662–666, 2005)

Abbreviations

FHR, fetal heart rate
GR, glucocorticoid receptor
LTV, long-term variation
MHR, maternal heart rate
SCN, suprachiasmatic nuclei
STV, short-term variation

In cases of threatened preterm delivery, synthetic glucocorticoids are administered antenatally to enhance fetal lung maturation. A meta-analysis by Crowley (1) showed that antenatal glucocorticoids not only reduced the incidence of respiratory distress syndrome by 50% but also the incidences of neonatal mortality, periventricular hemorrhage, and necrotizing enterocolitis. In 1994, this led to a recommendation by the National Institutes of Health to routinely administer either betamethasone or dexamethasone to all pregnant women at risk of preterm delivery before 34 wk of gestation (2). We have previously shown a transient reduction in fetal heart rate variation, breathing, and body movements 2 d after the first dose of betamethasone was administered (d 2) with a return to normal values 2 d later (3). It is not known what the effects were throughout d 2, *i.e.* whether

diurnal rhythmicity is affected or not. Although our findings could be suggestive of fetal hypoxemia, we did not find any changes in Doppler flow velocity waveforms in the uterine and umbilical arteries after betamethasone administration, nor did we find increased occurrence of fetal heart rate decelerations or a reduction in fetal eye movements (3,4). We concluded that the observed effects were more likely the result of a glucocorticoid receptor mediated process in the fetal brain.

Diurnal rhythms of FHR and its variation, body and breathing movements exist in the second half of pregnancy (5–8). FHR peaks during the day and minimum values are reached between 0200 and 0600 h, whereas FHR variation reaches lowest values between 0600 and 1200 h and peaks around 2400 h (6). The diurnal rhythm of fetal body movements is characterized by a low incidence in the morning hours and an increase in the late evening and night (7). At 30–31 wk gestation, fetal breathing movements show a distinct diurnal pattern. Lowest values (incidence of 10–12%) were found during daytime. After the ingestion of meals, which resulted in maternal glucose peaks, the incidence of breathing movements

Received February 24, 2003; accepted February 24, 2004.

Correspondence: S.V. Koenen, M.D., Ph.D., Department of Perinatology and Gynecology, University Medical Center Utrecht (HP F05.829), P.O. Box 85500, 3508 GA, Utrecht, The Netherlands; e-mail: stevenkoenen@planet.nl

DOI: 10.1203/01.PDR.0000159762.50504.1F

rose significantly during the second and third hours after the meal (incidence of 40–45%). After the third meal at 1800 h, breathing movements increased but did not return after the third hour to low levels. In contrast, they fell to about 20% and subsequently increased steadily to reach maximum values between 0400 and 0700 h (8). Fetal diurnal rhythms are to a large extent driven by the mother, and maternal cortisol has been postulated as a possible messenger in the maternal-fetal interrelationship of diurnal rhythms (9). Therefore, antenatal betamethasone administration could have an effect on fetal diurnal rhythms either by suppressing maternal adrenal gland activity or by a direct effect on the fetal brain.

The aim of the present study is to examine the effect of betamethasone on the normally present diurnal rhythms of FHR and its variation, breathing and body movements on the second day (d 2) after the first gift of the steroid.

METHODS

Subjects. Sixteen pregnant women at 26–32 wk of gestation at risk for preterm delivery were enrolled in the study after informed consent was obtained. Exclusion criteria were signs of intrauterine infection, or treatment with tocolytic drugs. The patients received two doses of betamethasone (12 mg, 24 h apart; Celestone Chronodose, Schering-Plough, Kenilworth, NJ) to enhance fetal lung maturation. The first injection was given on d 0. On the second day after the first injection (d 2), the study protocol started. All patients were in a stable clinical condition during the study period on d 2 and 3. Indications for steroid administration were intrauterine growth restriction with or without preeclampsia (IUGR, fetal biometry <5th centile, $n = 3$), placenta previa or other causes of vaginal blood loss ($n = 7$), and premature rupture of the membranes (PROM, $n = 6$). The study protocol was approved by the Institutional Review Board at our hospital.

Time line. Starting on d 2, 1-h recordings of fetal heart rate and movements were made simultaneously. Recordings began at 0800, and continued at 0900, 1200, 1600, 1700, 2200, and 2300 h and at 0800 and 0900 h on the next day (d 3). All subjects were exposed to a natural dark-light cycle, and the sleep-wake ratio was similar in all women. Timing of meals was similar in all patients (0730, 1300, and 1800 h), and contents were not standardized. Four to six days later (average, 4.3 d), control recordings were made at 0900, 1600, and 2200 h.

Methods. FHR was recorded using a Hewlett Packard 8040-A cardiotocograph (Boeblingen, Germany) with the woman in a semirecumbent position and was analyzed numerically by a computer running the System 8000 program (Sonicaid, Oxford Instruments Plc, Eynsham, Witney, UK). With this program, basal FHR (bpm, beats per minute) and pulse-interval differences were determined (10). Long-term FHR variation (LTV, ms) was calculated as the average of 1-min pulse-interval differences, whereas short-term FHR variation (STV, ms) was calculated as the average of 1/16-min pulse-interval differences.

Fetal body and breathing movements were visualized with the use of a linear-array real-time ultrasound transducer (Model SSD 620, Aloka Co., Tokyo, Japan; probe 3.5 MHz) and scored on-line into a personal computer (3). Tonic movements of the trunk occurring within 1 s of each other were regarded to be a single burst of movement. The following parameters were calculated per 1-h recording: the incidence (percentage of time spent moving) and the incidence of breathing movements (percentage of time spent breathing).

In nine women, maternal heart rate (MHR) was recorded on both d 2 and the control day using electrodes that were placed on the chest. Pulse intervals were stored into a personal computer for off-line analysis by the system 8000 system, yielding data for both MHR and its variation (LTV, STV).

Blood samples. To investigate the diurnal rhythms of maternal ACTH and cortisol, blood samples were obtained from five patients. Before the first betamethasone injection, a baseline blood sample was taken (d 0). On d 2, blood samples were taken at 0730, 1130, 1530, and 2330 h and the following morning (d 3) again at 0730 h. Four to six days later, when the control recordings were made in these patients, blood samples were taken according to the same time schedule. ACTH and cortisol were determined using commercially available kits. The baseline (d 0) sample was taken before betamethasone administration at one of the five time points (depending on the clinical situation) to compare it with its corresponding time points on d 2 and the

control day. This made it possible to calculate the absolute level of maternal ACTH and cortisol suppression.

Statistics. All data are presented as mean $\pm$ SEM. To test within-group differences on d 2, one-way repeated measures ANOVA was used followed by Dunnett's posthoc test. In this analysis, the seven time points on d 2 were used. To compare d 2 with the control day, two groups were distinguished: maternal and fetal recordings on d 2 (group 1) versus maternal and fetal recordings on the control day (group 2). Between-group differences were tested using two-way repeated measures ANOVA (with Mauchly correction). For statistical reasons only, three time points were used in the analysis; 0900, 1600, and 2200 h. To test differences within each group between the morning recordings (0900 h) and evening recordings (2200 h), the paired Student's *t* test was used. Where appropriate, Pearson's correlation coefficient was calculated. With all tests, two-tailed *p* values < 0.05 were considered to indicate statistical significance.

RESULTS

All 16 women completed the study protocol on d 2 and 3. Seven women delivered before the control recordings could be made 4–6 days later. Blood samples on d 2 and 3 and on the control day were obtained from five women.

Fetal parameters on d 2. On d 2, no normal diurnal patterns were found for FHR, LTV, STV, and body and breathing movements (Fig. 1). Repeated measures ANOVA did not show any significant time course for these parameters in all 16 patients. Fetal body movements showed a diurnal rhythm (Fig. 1), but the measure did not attain statistical significance ($p < 0.10$). The increase in FHR from d 2 to d 3 and the simultaneous decrease in FHR variation are consistent with our previous findings, when we studied these parameters longitudinally over a 5-d period (3).

Fetal parameters on d 2 versus the control day. The three recordings made on the control day (0900, 1600, and 2200 h) were compared with the corresponding d-2 recordings ($n = 9$ women). For each parameter, the 0900-h values were similar on either day, and not significantly different (Fig. 2). However, the parameters showed a differential time course in the fetuses exposed to betamethasone (d 2) and when unexposed (control day). This is demonstrated in Table 1 by a significant time $\times$ group effect (FHR, LTV, STV, trend for body movements) or main group effect (breathing movements). On the control day, body movements, breathing movements, LTV, and STV showed an increase and FHR a decrease with time, and their 2200-h values differed significantly from the 0900-h values

Figure 1. Effect of betamethasone on fetal body movements (a), breathing movements (b), FHR (c), and LTV (d) on the second and third day after steroid administration. Presented are the mean values (SEM) at each of the nine time points ($n = 16$).

Figure 2. Fetal body (a) and breathing movements (b), FHR (c), and LTV (d), on d 2 (closed markers) and the control day (open markers). Presented are the mean values (SEM) at each of the three time points ($n = 9$). *Significantly different from morning value. $p < 0.01$ for all except FHR ($p < 0.05$).

(Fig. 2). These normal diurnal patterns were completely absent on d 2.

Furthermore, a significant relationship was found between gestational age and the difference (delta) in the incidence of body movements between the 1600- and 2200-h recordings on d 2 ($R = -0.89$, $p < 0.01$) but not on the control day (Fig. 3). In other words, in older fetuses the evening rise in body movements was suppressed by betamethasone, whereas younger fetuses displayed a normal diurnal rhythm of body movements with an increased incidence in the evening.

Maternal heart rate. MHR showed a normal diurnal pattern on d 2, with highest values in the morning and lowest values in the evening, which was similar to and not statistically different from the diurnal pattern on the control day (Fig. 4, Table 1). Posthoc analysis showed significant differences between the morning (0900 h) and evening values (2200 h, $p < 0.01$) on either day. Furthermore, no significant differences were found between the morning recordings on d 2 and 3. Similar results, though in opposite direction, were found for MHR variation (Fig. 4, Table 1).

ACTH and cortisol. Both the ACTH and cortisol rhythms were completely suppressed on d 2 (Fig. 5). Although on the control day absolute levels of both hormones were still reduced by $45.1 \pm 4.3\%$ and $59.2 \pm 10.2\%$ for ACTH and cortisol, respectively, both hormones showed a normal diurnal rhythm with highest values in the early morning and lowest values around midnight (Fig. 5, Table 1).

DISCUSSION

This study demonstrates that betamethasone given to pregnant women transiently abolishes the fetal diurnal rhythms of fetal heart rate and its variation, breathing and body movements. This is mainly the result of suppression of the expected rise in body and breathing movements, and of heart rate variation in the afternoon and evening. Values of fetal parameters during the morning recordings were similar on d 2 and 4–6 d thereafter, but differed at the other time points (Fig. 2). Four to six days later the rhythms are restored. Betamethasone did not affect the maternal diurnal rhythms of heart rate and its variation. Although maternal diurnal rhythms of cortisol and ACTH are absent on d 2 and absolute

values are considerably reduced, both have resumed their normal diurnal patterns 4–6 d later.

Before starting the present study, we hypothesized that the previously found reduction in FHR variation and body and breathing movements 2 d after betamethasone administration is caused by overall suppression of these parameters on d 2 (3). Therefore, we expected to find differences in these parameters between the study and control days for each parameter at each time point. However, these differences were only found for the afternoon and evening values and not for the morning hours (Fig. 2). This suggests that the previously found reductions in FHR variation and fetal movements on the second day after betamethasone administration are caused by the suppression of the diurnal rhythms of these parameters (3). Furthermore, we also demonstrated that the suppression of the diurnal rhythm of body movements was gestational age dependent (Fig. 3).

Diurnal rhythms of FHR and its variation, body and breathing movements, exist in the second half of pregnancy (5–8). Diurnal rhythms are assumed to be driven by a circadian pacemaker, the suprachiasmatic nuclei (SCN) in the hypothalamus (9). Reppert (9) demonstrated in the rat that the fetal SCN were synchronized by the maternal SCN. It is unclear how this entrainment takes place. Several mediators have been postulated as possible “Zeitgeber” such as hormones (melatonin, cortisol, prolactin), food-intake, and body temperature (11). Taylor *et al.* (12) even suggested uterine contractions as a possible pathway of communication between mother and fetus. Cortisol has long been suspected to play an important role as a possible messenger in the maternal-fetal interrelationship. It shows a robust 24-h rhythm that is present throughout pregnancy (13). Patrick *et al.* also demonstrated a 24-h rhythm for estradiol (E3) in maternal serum, which reflects diurnal activity of the fetal hypothalamic pituitary adrenal axis (HPA). The fetal adrenal produces DHEAS that is converted in the placenta to E3. Interestingly, the E3 rhythm is inversely related to the cortisol rhythm. Previous studies demonstrated that administration of exogenous glucocorticoids to pregnant women results in abolished diurnal rhythms of maternal cortisol, ACTH, and E3 and fetal activity but does not affect the diurnal rhythms of maternal and fetal heart rate (14–16). The present data clearly show that betamethasone given to pregnant women has a pronounced effect on the diurnal patterns of fetal body movements, breathing movements, FHR, and FHR variability (Fig. 1). This effect is of short duration inasmuch as diurnal rhythms have returned to normal just a few days later (Fig. 2). The question remains whether betamethasone directly affects the fetal SCN or whether it interferes with other centers in the fetal brain that are involved in motor and breathing activity, such as the raphe nuclei (RN), the locus ceruleus (LC), and the nucleus of the solitary tract (NTS) (17,18). In the adult rat and fetal sheep, glucocorticoid receptors (GR) have been identified in all of these nuclei including the SCN (19–21). GR have a very high affinity for synthetic glucocorticoids and their occupancy suppresses neural activity (19,20). The intrauterine environment is influenced by several rhythmic hormones of the mother (melatonin, cortisol, prolactin). All of these have been studied in animal experiments (rats, hamsters, mice) as the possible mediator for fetal circadian entrainment. However,

Table 1. Overview of the results of the two-way repeated measures ANOVA for fetal and maternal parameters

Parameter	Effect of time		Effect of time $\times$ group		Effect of group	
	F(2,15)	p Value	F(2,15)	p Value	F(1,16)	p Value
FHR (bpm)	0.5	NS	5.3	<0.01	0.1	NS
LTV (ms)	8.8	<0.001	3.9	<0.05	0.9	NS
STV (ms)	4.8	<0.05	3.3	0.05	0.3	NS
%BM	3.0	0.06	2.1	0.09	1.5	NS
%BrM	2.9	0.07	1.8	NS	7.6	<0.05
MHR (bpm)	24.4	<0.0001	0.4	NS	0.1	NS
MHR-LTV (ms)	6.6	<0.02	0.5	NS	1.7	NS
MHR-STV (ms)	10.6	<0.005	1.3	NS	1.6	NS
	F(2,7)	p Value	F(2,7)	p Value	F(1,8)	p Value
Cortisol (umol/L)	4.8	<0.05	4.6	<0.05	7.7	<0.05
ACTH (ng/L)	1.4	NS	1.4	NS	3.5	0.10

For both $n = 9$, except for cortisol/ACTH ($n = 5$). Shown are fetal heart rate (FHR), long-term variation (LTV), short-term variation (STV), incidence of body movements (%BM), incidence of breathing movements (%BrM), maternal heart rate (MHR) and its long-term (LTV) and short-term (STV) variation, cortisol, and ACTH.

Figure 3. Relationship between gestational age and the difference (delta) in the incidence of fetal body movements between the 1600- and 2200-h recordings on d 2 (closed markers, $R = -0.89$; $p < 0.01$) and the control day (open markers, NS).

maternal pinealectomy (removal of melatonin) alone did not abolish maternal coordination of fetal circadian rhythms (9). Furthermore, maternal adrenalectomy or removal of the pituitary (in separate experiments) did not abolish the diurnal rhythm of metabolic activity in the fetal rat SCN (9). In other words, there most probably is not one single maternal signal that entrains the fetal circadian clock. However, this does not mean that cortisol is not an important “Zeitgeber.” An excess of glucocorticoids, like in the present study, could alter fetal diurnal rhythms directly through the SCN when all the other “Zeitgebers” are still present and active. It is also possible that the temporary reduction in fetal body and breathing movements on d 2 results from direct suppression of the brain centers of motor and breathing activity (RN, LC, NTS), resulting in disruption of the fetal diurnal rhythms of body and breathing movements and, subsequently, FHR variation. If this were the case, the suppression of movements should also have been present in the early morning recordings as well, but this was not the case (Fig. 2). The fact that the suppression of the diurnal rhythm of body movements is dependent on gestational age (Fig. 3) suggests that in younger fetuses glucocorticoid receptors might not be fully functional.

Figure 4. Effect of betamethasone on maternal heart rate (MHR, upper graph) and long-term variation (MLTV, lower graph) on both d 2 and 3 (closed markers) after betamethasone administration and the control day (open markers). Data presented as mean $\pm$ SEM ($n = 9$).

If the maternal cortisol rhythm is a major time cue this cannot be disproved by the present data. Although the maternal ACTH and cortisol levels are still suppressed considerably 6–8 d after the first steroid gift, the rhythms have returned with highest values in the early morning and lowest values around midnight. This occurs simultaneously with the return of the fetal diurnal rhythms of heart rate variation and movements.

With respect to clinical significance, it is clear that our results have direct consequences for fetal surveillance in high-risk pregnancies. Not only should clinicians know of the reductions of fetal movements and heart rate variation on the second day after glucocorticoid administration, they should also be aware of its effect on the diurnal rhythm of these parameters. For fetal monitoring and especially for assessing trends in fetal heart rate variation and movements

Figure 5. Maternal cortisol (upper graph) and ACTH (lower graph) on d 2 and 3 (closed markers) after betamethasone administration, and the control day (open markers). Presented are the mean values (SEM) at each of the five time points ($n = 5$).

with time (e.g. in case of IUGR), early morning recordings should be preferably used in the period around glucocorticoid administration. Secondly, it is unclear what the transient disturbance of these fetal diurnal rhythms means for the development of postnatal diurnal rhythms. It is generally assumed that entrainment of the circadian clock *in utero* helps the fetus to prepare for life in the outside world. Interruption of this entrainment process could impede postnatal diurnal rhythm development (11).

In conclusion we have demonstrated that betamethasone given to pregnant women at risk of preterm delivery transiently abolishes the diurnal rhythms of fetal body movements, breathing movements, FHR and its variation. The previously found reduction in FHR variation and body and breathing movements 2 d after betamethasone administration can be explained by the suppression of the diurnal rhythms of these parameters.

REFERENCES

1. Crowley PA 1995 Antenatal corticosteroid therapy: a meta-analysis of the randomized trials, 1972 to 1994. *Am J Obstet Gynecol* 173:322-355
2. [No authors listed] 1995 Effect of corticosteroids for fetal maturation on perinatal outcomes. NIH Consensus Development Panel on the Effect of Corticosteroids for Fetal Maturation on Perinatal Outcomes. *JAMA* 273:413-418
3. Derks JB, Mulder EJ, Visser GH 1995 The effects of maternal betamethasone administration on the fetus. *Br J Obstet Gynaecol* 102:40-46
4. Cohlen BJ, Stigter RH, Derks JB, Mulder EJ, Visser GH 1996 Absence of significant hemodynamic changes in the fetus following maternal betamethasone administration. *Ultrasound Obstet Gynaecol* 8:252-255
5. de Vries JJ, Visser GH, Mulder EJ, Prechtl HF 1987 Diurnal and other variations in fetal movement and heart rate patterns at 20-22 weeks. *Early Hum Dev* 15:333-348
6. Visser GH, Goodman JD, Levine DH, Dawes GS 1982 Diurnal and other cyclic variations in fetal heart rate near term. *Am J Obstet Gynecol* 142:535-544
7. Patrick J, Campbell K, Carmichael L, Natale R, Richardson B 1982 Patterns of gross fetal body movements over 24-hour observation intervals during the last 10 weeks of pregnancy. *Am J Obstet Gynecol* 142:363-371
8. Patrick J, Campbell K, Carmichael L, Natale R, Richardson B 1980 Patterns of human fetal breathing during the last 10 weeks of pregnancy. *Obstet Gynecol* 56:24-30
9. Reppert SM 1992 Pre-natal development of a hypothalamic biological clock. In: Swaab DF, Hofman MA, Mirmiran M, David R, van Leeuwen FW (eds) *Progress in Brain Research: The Human Hypothalamus in Health and Disease*. Elsevier Science Publishers, Amsterdam, pp 119-133
10. Dawes GS, Moulden M, Redman CW 1991 System 8000: computerized antenatal FHR analysis. *J Perinat Med* 19:47-51
11. Mirmiran M, Kok JH, Boer K, Wolf H 1992 Perinatal development of human circadian rhythms: role of the foetal biological clock. *Neurosci Biobehav Rev* 16:371-378
12. Taylor NF, Martin MC, Nathanielsz PW, Seron-Ferre M 1983 The fetus determines circadian oscillation of myometrial electromyographic activity in the pregnant rhesus monkey. *Am J Obstet Gynecol* 146:557-567
13. Patrick J, Challis J, Campbell K, Carmichael L, Natale R, Richardson B 1980 Circadian rhythms in maternal plasma cortisol and estradiol concentrations at 30 to 31, 34 to 35, and 38 to 39 weeks' gestational age. *Am J Obstet Gynecol* 136:325-334
14. Challis J, Patrick J, Richardson B, Tevaarwerk G 1981 Loss of diurnal rhythm in plasma estrone, estradiol, and estriol women treated with synthetic glucocorticoids at 34 to 35 weeks' gestation. *Am J Obstet Gynecol* 139:338-343
15. Arduini D, Rizzo G, Parlati E, Giorlandino C, Valensise H, Dell'Acqua S, Romanini C 1985 Modifications of ultradian and circadian rhythms of fetal heart rate after fetal-maternal adrenal gland suppression: a double blind study. *Prenat Diagn* 6:409-419
16. Lunshof S, van Someren EJ, Kortes-van Hoffen G, Wolf H, Boer K 2000 Lack of relationship between diurnal rhythms in fetal heart rate and maternal cortisol concentration. *Am J Obstet Gynecol* 183:460-461
17. Jacobs BL 1985 Overview of the activity of brain monoaminergic neurons across the sleep-wake cycle. In: Wauquier A, Monti JM, Gaillard JM, Radilovacki M (eds) *Sleep: Neurotransmitters and Neuromodulators*. Raven Press, New York, pp 1-14
18. Long S, Duffin J 1986 The neuronal determinants of respiratory rhythm. *Prog Neurobiol* 27:101-182
19. Kloet ER de, Rosenfeld P, van Eekelen JAM, Sutanto W, Levine S 1988, Stress, glucocorticoids and development. In: Boer GJ, Feenstra MGP, Mirmiran M, Swaab DF, van Haaren F (eds) *Progress in Brain Research*. Elsevier Science Publishers BV, Amsterdam, pp 101-120
20. de Kloet ER, Reul JM, Sutanto W 1990 Corticosteroids and the brain. *J Steroid Biochem Mol Biol* 37:387-394
21. McDonald TJ, Myers DA, Nathanielsz PW 1993 Distribution of type II glucocorticoid receptor in selected areas of the brain of the fetal sheep at 120 days of gestation. *J Physiol* 459:33P