0013-7227/01/$03.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 86(12):5981–5987

Neuroactive Ring A-Reduced Metabolites of Progesterone in Human Plasma during Pregnancy: Elevated Levels of 5α-Dihydroprogesterone in Depressed Patients during the Latter Half of Pregnancy

BEVERLEY E. PEARSON MURPHY, SUSANNE I. STEINBERG, FEN-YUN HU, AND CYNDIE M. ALLISON

Psychoendocrinology Unit, Allan Memorial Institute (B.E.P.M., F.-Y.H., C.M.A.), Departments of Psychiatry (B.E.P.M., S.I.S., C.M.A.), Obstetrics & Gynecology (B.E.P.M.), and Medicine (B.E.P.M.), McGill University, Montréal, Québec, Canada H3G 1A4

Progesterone and its 5α reduced metabolite, 5α-dihydroprogesterone, rise greatly in pregnancy. Both are known to have anesthetic properties, as do a number of other ring A-reduced progesterone metabolites. The possible significance of these steroids with respect to the mood changes that are common in pregnancy and in the puerperium has not been explored. In this study, pregnenolone, progesterone, and five neuroactive progesterone metabolites: the 5α and 5β dihydroprogesterones (DHP), and three tetrahydroprogesterones (THP)—3α,5α-THP, 3β,5β-THP, and 3β,5α-THP—were studied at various stages of pregnancy and in the early postpartum period.

Levels of all of the steroids rose greatly during pregnancy ($P < 0.001$), being highest for progesterone (562-fold the follicular level), 5α-DHP (161-fold), 3β,5α-THP (56-fold), 3α,5α-THP (37-fold), pregnenolone (30-fold), 5β–DHP (16-fold) and 3β,5β-THP (16-fold) at 37 wk of gestation. During the period 2–7 d postpartum, the level of progesterone fell precipitously, whereas those of pregnenolone and the metabolites fell more slowly and mean levels were still elevated compared with follicular levels 2 wk after delivery. By 7 wk postpartum, only 3α,5α-tetrahydroprogesterone and 3β,5β-tetrahydroprogesterone remained slightly elevated ($P \leq 0.012$ and 0.007, respectively).

Mean levels of the progesterone metabolites tended to be higher in depressed patients compared with controls, and this difference reached significance for 5α-dihydroprogesterone both at 27 wk ($P = 0.04$) and at 37 wk ($P = 0.02$) of gestation (combined, $P = 0.003$).

These results show that all five of these metabolites rise markedly during pregnancy and suggest that alterations in progesterone metabolites may be involved in the mood changes of pregnancy and the puerperium. (*J Clin Endocrinol Metab* 86: 5981–5987, 2001)

THE TERM "neuroactive steroids" as used here refers to steroids that are active on neural tissue and that may be synthesized endogenously in the brain itself (neurosteroids) or elsewhere in the body. Pregnenolone is the precursor of all mammalian steroid hormones, being produced by the adrenal cortex, the ovary, the testis, the placenta, and by the brain itself. These tissues produce a variety of corticoids, progestins, androgens, and estrogens, and also the lesser known group of "anesthetic steroids" (neuroactive A-ring reduced steroids, NARS), whose physiological significance is still unknown.

It has been recognized for more than 50 yr that, when injected as a bolus, many of the ring A-reduced metabolites of progesterone have potent direct (nongenomic) effects on the brains of mammals, and indeed are among the most powerful anesthetics known (1–4); however their blood levels are low, and their measurement is difficult. Interest in these compounds increased recently, when some of them, such as allopregnanolone (for structures and abbreviations of the steroids measured, see Table 1) were shown to bind stereoselectively and with high affinity to receptors for γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in brain; they are thought to affect cognition, memory, and mood (for reviews, see Refs. 5 and 6). Others may be GABA receptor antagonists (7, 8). The pathways of steroid synthesis and metabolism are complex, so that changes in the availability of one steroid hormone may alter both the production and the route of metabolism of others through competition for enzymes and induction of enzymes. This means that lack of direct correlation between alterations of the principal steroid hormones and pathology does not preclude a major etiological role for steroids.

Because progesterone rises more than 10-fold during pregnancy compared with the luteal phase of the menstrual cycle, it is pertinent to examine its neuroactive metabolites with respect to any physiological changes that may occur during and following pregnancy.

The aim of this study was to document levels of pregnenolone and progesterone and five of its neuroactive metabolites during pregnancy and the puerperium, using a method described recently (9). Pregnenolone, the precursor of progesterone, was also measured. In addition, patients with no previous history of mental problems who became depressed during pregnancy were also studied and showed

Abbreviations: DHP, Dihydroprogesterone; GABA, γ-aminobutyric acid; Ham-D, Hamilton Depression Scale; NARS, neuroactive A-ring reduced steroids; THP, tetrahydroprogesterone.

TABLE 1. Structure and anesthetic activity of some neurosteroids and other anesthetic agents

Compound	Abbreviation	Anaesthetic potency (μmol/kg)	Reference
Pregn-4-ene-3,20-dione (progesterone)	P	283	Gyermek and Soyka, 1975
1. 5α-Pregnane-3,20-dione (5α-dihydro-P)	5α-DHP	202[a]	"
4. 3α-Hydroxy-5α-pregnan-20-one (3α,5α-tetrahydro-P, allopregnanolone)	3α,5α-THP[b]	9	"
5. 3β-Hydroxy-5α-pregnan-20-one (alloepipregnanolone)	3β,5α-THP	38*[a]	Selye, 1942
2. 5β-Pregnane-3,20-dione	5β-DHP[b]	38	Figdor, 1957
3. 3β-Hydroxy-5β-pregnan-20-one (epipregnanolone)	3β,5β-THP	47	"
Thiopental-Na		68	"
Ketamine		47	Gyermek and Soyka, 1975
3β-Hydroxypreg-5-ene-20-one (pregnenolone)	P5	Inactive	Figdor, 1957

All potencies are for compounds injected iv into mice, except as indicated by an *asterisk*. *Asterisk*, dose by ip injection required for loss of the righting reflex in four of six partially hepatectomized female rats. Numbers 1–5 denote the order of elution as indicated in Fig. 1.

[a] Also convulsant.

[b] $GABA_A$ receptor agonist.

elevations of 5α-dihydroprogesterone. These data were reported in part at the Annual Meeting of The Endocrine Society (10).

Subjects and Methods

Subjects

The patients in this study were drawn from a group of 203 healthy women, with no previous history of mental problems of any kind and not in high-risk obstetrical categories, recruited during pregnancy and followed to delivery and for several weeks following. Most were seen at 26–28 wk gestation, 36–38 wk gestation, at 2–7 d after delivery, and at 2 wk (13–18 d) and 6 wk (42–58 d) postpartum. A few were seen in early pregnancy, at 11–19 wk. Data for 155 women regarding changes in mood, life stress measures, plasma tryptophan concentrations and platelet imipramine binding will be presented elsewhere (Steinberg, S. I., L. Annable, S. N. Young, J. Martial, N. Liyanage, D. Ramdoyal, S. Mainville, and F. Roule, in preparation). When seen, blood samples were obtained if possible.

Each subject underwent a physical examination and a structured psychiatric clinical interview (11) to exclude physical and psychiatric illness. Results for a limited number of those remaining physically well throughout and immediately following pregnancy are reported here, as well as those for women who became depressed during or following pregnancy. Because samples were not available for all women at all visits, this is not strictly a longitudinal study, although in some cases subjects were studied more than once. Samples for nonpregnant women in the follicular and/or luteal phase were collected from healthy women, with regular menstrual periods, who were not taking medications.

For the pregnant patients, inclusion criteria were: age between 21 and 45 yr; in good physical health; pregnancy confirmed by the obstetrician; involved in a stable relationship with the father of the child. Excluded were: women in high risk obstetrical categories; those with a current or previous documented DSM III-R Axis I diagnosis (12); those with a requirement for medication other than the usual vitamin preparations prescribed during pregnancy. All subjects gave written informed consent to participate in the study, which was approved by the Research Ethics Board of St. Mary's Hospital, where the patients delivered.

Psychological assessments

Various rating scales were completed at each visit. Those used here included the Hamilton Depression Rating Scale (Ham-D) (13), the Maternity Blues Scale of Kennerley and Gath (14), and the SCL-90-R scale of Derogatis *et al.* (15). The Ham-D was modified for pregnancy so that the items concerning weight loss and somatic manifestations such as backache, gastrointestinal disturbances, and urinary frequency, which are common in pregnancy, were not scored. Control subjects were chosen who had a Ham-D score ≤ 7.

The criteria for maternity blues used here required a score of 12 or greater on the 28-item Blues scale within the period of 2–7 d following delivery; controls were chosen with a score ≤ 7.

Mood in nonpregnant subjects was assessed using the self-rating SCL-90 questionnaire, which we have found to correlate well with the Ham-D. None had a depression rating above 6. Patients in early pregnancy were assessed using a similar scale modified for pregnancy.

Patient sampling

Among the 12 subjects studied at 12–19 wk, none met the criteria for depression. At 26–28 wk gestation, samples were available for nine depressed subjects as defined by a modified Ham-D ≥ 13. They were compared with 12 control subjects selected randomly from the available samples for women who had Ham-D's less than 6. At 36–38 wk, samples on six depressed subjects were available; they were compared with nine controls chosen as above. During the early postpartum period samples on five patients with Blues were available, and were compared with ten controls, while at 2 wk postpartum, five depressed patients were compared with 11 controls. Two of the patients sampled were depressed both at 27and at 37 wk gestation, and also suffered from the "blues"; one other was depressed only by 37 wk and also suffered from "blues." Unfortunately, many patients failed to arrive for their postpartum visits, and at 7 wk samples were only available on five controls. Fewer postpartum samples were usable than expected because some of the samples had insufficient volume for the larger sample size required in nonpregnant subjects (preferably 6 ml of plasma, for duplicate assays).

Heparinized blood samples were taken in the morning at 0900–1000 h, and the plasma was separated, frozen, and stored at -20 C until assayed. Six samples were also taken without heparin and the results compared; no differences were observed.

Determination of levels of progesterone and its metabolites

This method has recently been described in detail (9). Briefly, after addition of tracer tritiated progesterone (about 3000 cpm), an aliquot of plasma (0.50 ml in early pregnancy, 0.10 ml in late pregnancy, and 1.0–3.0 ml postpartum) was extracted with toluene, and the organic phase dried. The extract was redissolved in methylene chloride and passed through HPLC. The exclusion volume was approximately 0.5 ml. The eluate was split, one-third collected into counting vials for determination of recovery, and the remaining two-thirds was collected into 50 assay tubes for RIA. Because of the very high progesterone levels, the tubes containing the progesterone were diluted so that only 1/10 was assayed. Each sample was carried through the whole procedure at least twice, and the mean values analyzed.

Recoveries of tracer progesterone averaged about 50%. For 28 samples, a tracer of tritiated 3α,5α-THP was run simultaneously; the recovery averaged 50.5% for progesterone and 50.7% for 3α,5α-THP, and the SD for the two tracers was $\pm$ 2.9%. The coefficients of variation of the final values for the metabolites varied from $\pm$ 11% for levels over 40 ng/ml to $\pm$ 20% for those below 0.5 ng/ml. Sensitivity was 7 pg for 3β,5α-THP, 33 pg for 3α,5α-THP, and 20 pg for the others.

Because pregnenolone eluted exactly with 3β,5α-THP and cross-reacted 6% as strongly, it was determined separately by an RIA that did

not cross-react with the other steroids, and the value for 3β,5α-THP was corrected accordingly (9).

Mean values for depressed (or blues) and control groups were compared using *t* test, or, where the standard deviations differed significantly, the Mann-Whitney nonparametric test or Welch's approximation.

Results

Figure 1 shows a typical pattern obtained at 32 wk gestation. All of the metabolites measured eluted before progesterone. In addition to the seven steroids listed above, we found in all samples cross-reactive material eluting before 5α-DHP, which also rose during pregnancy. This was resolved into at least eight peaks. When summed as progesterone equivalents, their total averaged about 12% that of progesterone in the luteal phase of the menstrual cycle, 9% in early pregnancy, 7% at 26–28 wk, 6% at 36–38 wk, but relatively more (200–500%) in women in the follicular phase, and by 2 wk postpartum. There was also a small unidentified peak consistently detectable immediately following the 5α-DHP peak, which is just visible in the graph. This peak was also relatively larger in nonpregnant subjects.

All of the steroids measured, as well as the unidentified material, rose considerably during pregnancy and fell following delivery (Fig. 2 and Table 2). In control subjects, by 37 wk, progesterone had increased from nonpregnant luteal levels of about 30 nmol/liter to 657 nmol/liter, a 22-fold rise, whereas pregnenolone rose from 4 nmol/liter luteal to 68 nmol/liter, a 17-fold rise. The highest of the progesterone metabolites measured was 5α-DHP, which rose from about 1.5 nmol/liter to 29 nmol/liter, an approximately 20-fold rise. 5β-DHP rose from luteal values of about 0.5 nmol/liter to 2.9 nmol/liter, 3β,5β-THP from 0.4 nmol/liter to 2.2 nmol/liter, 5α,3α-THP from 2.4 nmol/liter to 14 nmol/liter, and 3β,5α-THP from 0.5 nmol/liter to 5 nmol/liter-all 5- to 10-fold. Expressed as change from follicular levels, progesterone rose 562-fold, 5α-DHP 161-fold, 3β,5α–THP 56-fold, 3α,5α-THP 37-fold, pregnenolone 30-fold, and 5β-DHP and 3β,5β-THP 16-fold, by 37 wk of gestation. The unidentified material rose 27-fold.

After delivery (Fig. 2), progesterone fell very rapidly, averaging 1/60th the prepartum level at 2–7 d, whereas pregnenolone and the metabolites all fell more slowly so that at 2–7 d they averaged 1/5 to 1/10th the prepartum levels. Even by 13–18 d following delivery, although all the levels had fallen considerably further, all but progesterone itself were still significantly elevated ($P \leq 0.007$–0.03) compared with follicular levels. By 7 wk, the levels were not significantly different from follicular control levels, save for 3β,5β-THP ($P = 0.007$) and 3α,5α-THP ($P = 0.012$), which, although lower than at 2 wk postpartum, remained elevated, as did the unidentified material.

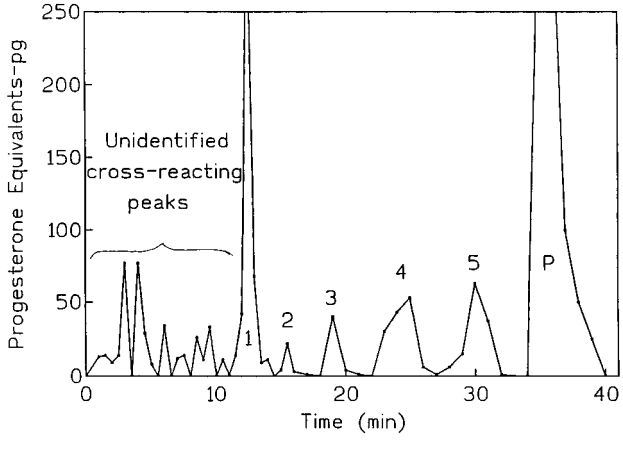

FIG. 1. The raw data of a chromatogram for a sample at 32 wk gestation are shown. In addition to the known steroids, at least eight other peaks are eluted before 5α-DHP, and one small one immediately after it. These remain to be identified. Numbers refer to steroids in Table 1.

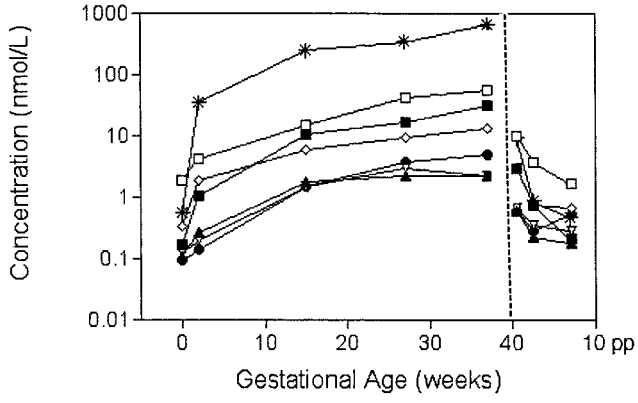

FIG. 2. Mean changes in steroid levels during and following pregnancy. Note log scale. * , Progesterone; □, pregenolone; ■, 5α-DHP; ◇, 3α,5α-THP; ○ = 3β,5α-THP; ▲, 5β-DHP; ▽, 3β,5β-THP. F, Follicular; L, luteal; wks, wk gestation; pp, postpartum. Delivery is represented by a *dotted line* at 40 wk. The "gestational age" beyond 40 wk is the first 10 wk of the postpartum period.

Among all the women studied, 7% met criteria for depression during pregnancy and the depression persisted postpartum in 4%; 25% experienced symptoms of "blues" as defined here during the week following delivery, while 7%, who were not depressed during pregnancy, developed postpartum depression.

Figure 3 compares the results for depressed and control subjects for samples taken at 26–28 wk and at 36–38 wk gestation. Four of the mean values for the metabolites at 26–28 wk were higher in the depressed group than those in the control group, but only that for 5α-DHP reached significance ($P = 0.04$). At 36–38 wk gestation, mean values again tended to be higher in the depressed group, but again only the difference for 5α-DHP was significant ($P = 0.02$). Values for two of the depressed patients (depressed at both ages) and three of the controls (not depressed at both ages) were obtained at both 27 and 37 wk gestation. The combined data, calculated as % mean control value, and taking the mean value at the two gestational ages for each patient studied more than once, gives a level of significance for 5α-DHP of $P = 0.004$ for the 13 depressed patients, and 18 controls (or, if log transformed, $P = 0.003$).

During the early postpartum period (2–7 d following delivery), samples on only five patients with "blues" were available and these were compared with ten controls. No significant differences were seen. At 13–18 d postpartum, no significant differences were found between the five depressed patients and 11 controls.

TABLE 2. Steroid levels (nmol/liter) and mood scores before, during, and following pregnancy (mean ± SD)

Steroid	Group	n	Ham-D or blues	5α-DHP	5β-DHP	3β,5β-THP	3α,5α-THP	3β,5α-THP	Preg	Prog
Follicular	Ctl	13	3 ± 2	0.19 ± 0.07	0.11 ± 0.06	0.12 ± 0.06	0.34 ± 0.20	0.10 ± 0.06	1.86 ± 0.90	0.53 ± 0.58
Luteal	Ctl	9	3 ± 2	1.07 ± 0.57	0.27 ± 0.27	0.20 ± 0.18	1.84 ± 1.18	0.14 ± 0.14	4.24 ± 2.36	34.9 ± 17.9
12–19 wk	Ctl	12	4 ± 2	10.4 ± 4.2	1.74 ± 1.17	1.47 ± 1.35	5.83 ± 4.13	1.49 ± 1.15	14.7 ± 8.4	246 ± 111
26–28 wk	Ctl	12	4 ± 2	17.1 ± 5.6	2.22 ± 1.22	2.92 ± 1.85	9.31 ± 3.32	3.74 ± 1.41	42.9 ± 32.5	335 ± 107
	Dep	9	18 ± 8	23.9[a] ± 8.4	2.81 ± 1.26	2.89 ± 1.32	11.7 ± 2.3	4.04 ± 1.75	42.3 ± 13.0	353 ± 148
36–38 wk	Ctl	9	3 ± 2	31.0 ± 9.5	2.30 ± 1.16	2.21 ± 1.37	13.5 ± 5.8	5.01 ± 2.63	57.3 ± 13.3	657 ± 178
	Dep	6	18 ± 3	46.6[b] ± 14.9	2.86 ± 2.03	2.21 ± 1.81	13.3 ± 3.5	8.30 ± 4.07	68.4 ± 22.2	718 ± 360
2–7 d pp	Ctl	11	2 ± 3	2.30 ± 1.54	0.61 ± 0.41	0.68 ± 0.35	2.94 ± 1.93	0.61 ± 0.21	9.85 ± 8.51	8.99 ± 6.62
	Blues	5	16 ± 2	1.98 ± 0.98	0.59 ± 0.27	1.03 ± 0.31	2.40 ± 0.76	0.96 ± 0.56	9.74 ± 3.71	5.79 ± 5.09
13–18 d pp	Ctl	11	2 ± 2	0.74 ± 0.57	0.22 ± 0.12	0.35 ± 0.24	0.76 ± 0.39	0.28 ± 0.24	3.73 ± 2.14	0.85 ± 0.55
	Dep	5	19 ± 1	0.38 ± 0.21	0.27 ± 0.19	0.40 ± 0.29	1.02 ± 0.79	0.20 ± 0.12	2.09 ± 0.90	1.04 ± 0.47
43–58 d pp	Ctl	5	4 ± 1	0.21 ± 0.05	0.18 ± 0.11	0.28 ± 0.14	0.66 ± 0.26	0.53 ± 0.35	1.68 ± 1.37	0.46 ± 0.21

Ctl, Control; Dep, depressed; Preg, pregnenolone; Prog, progesterone; pp, postpartum.
[a] $P = 0.04$.
[b] $P = 0.02$.

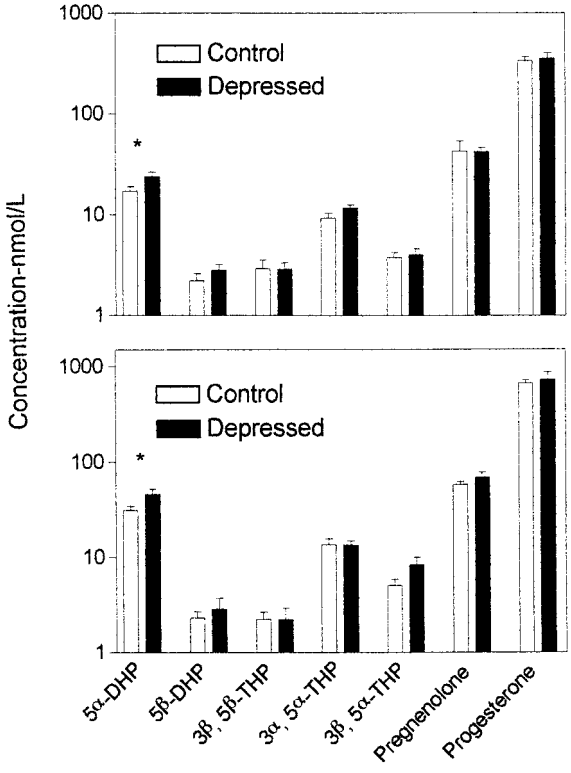

FIG. 3. Steroid levels (nmol/liter) (mean ± SE): *Top*, at 26–28 wk; and *Bottom*, at 35–38 wk gestation. *, $P \leq 0.05$.

Discussion

We have studied only five of about 20 NARS that may be pertinent (see Fig. 4). The NARS measured here were chosen for technical reasons, but it is potentially important and feasible to measure all of them. Because desoxycorticosterone levels rise in pregnancy, though to a lesser extent than progesterone (16), one would expect that its NARS also rise.

Our levels for progesterone are consistent with those in the literature, our mean value of 657 nmol/liter at 36–38 wk being comparable to values found by Tulchinsky *et al.* (17). We were unable to find data for pregnenolone in pregnancy.

In this study, the material that eluted very early on HPLC consisted of at least eight peaks, which although still not definitively identified, are probably lipoidal in nature—likely fatty acid esters of progesterone metabolites conjugated at the C3 position. This seems reasonable because these peaks 1) cross-react with our antibody, 2) rise through pregnancy, 3) are very nonpolar, and 4) appear to correspond to material eluted early when progesterone is metabolized by lymphocytes (18). Because our antibody was raised to a C3-linked antigen, the progesterone metabolites measured here with the addition of a fatty acid at the C3 position would be expected to cross-react. Such lipoidal steroids or "liposteroids" have been identified in bovine corpora lutea (19). This material expressed as progesterone equivalents is relatively large, amounting in late pregnancy to about 6% of the progesterone level, but to 200–500% of the progesterone level in follicular phase plasma. Similar amounts were seen in plasma of men and postmenopausal women (Murphy, B. E. P., and C. M. Allison, unpublished observations). Because the same compounds occur in men, and the testis produces very little progesterone, the main sources are probably adrenal and brain. Lipoidal derivatives of pregnenolone and some of its derivatives, including allopregnanolone, have been isolated from bovine corpora lutea (19, 20), and lipoidal derivatives of E2 have been found in human breast cancer cells (21). There is evidence that the C17 fatty acid esters of E2 are long-acting estrogens (22). The persistence of increased levels of all the free metabolites by 2 wk postpartum could be due to longer half-lives, slow breakdown of lipoidal compounds (which, however, also remained elevated), or to increased synthesis.

Our levels of 5α-DHP both in nonpregnant and pregnant subjects are lower—about 1/3—of those found by others, probably due to the greater precision of high performance liquid chromatography in separating steroids that interfere with the RIA, as discussed previously (9). During pregnancy, Parker *et al.* (23) reported that levels of 5α-DHP rose to 1/7 those of progesterone from 12–15 wk gestation, whereas at 35–41 wk, the ratio had risen to 1/5—values of 40 ± 20 ng/ml (111 nmol/liter). Dombroski *et al.* (24) showed that the high levels of 5α-DHP were attributable to high rates of production rather than to low rates of clearance, and that about 70% of 5α-DHP is cleared in extrahepatic tissues. Löfgren *et al.* (25) found that 5α-DHP fell before the onset of

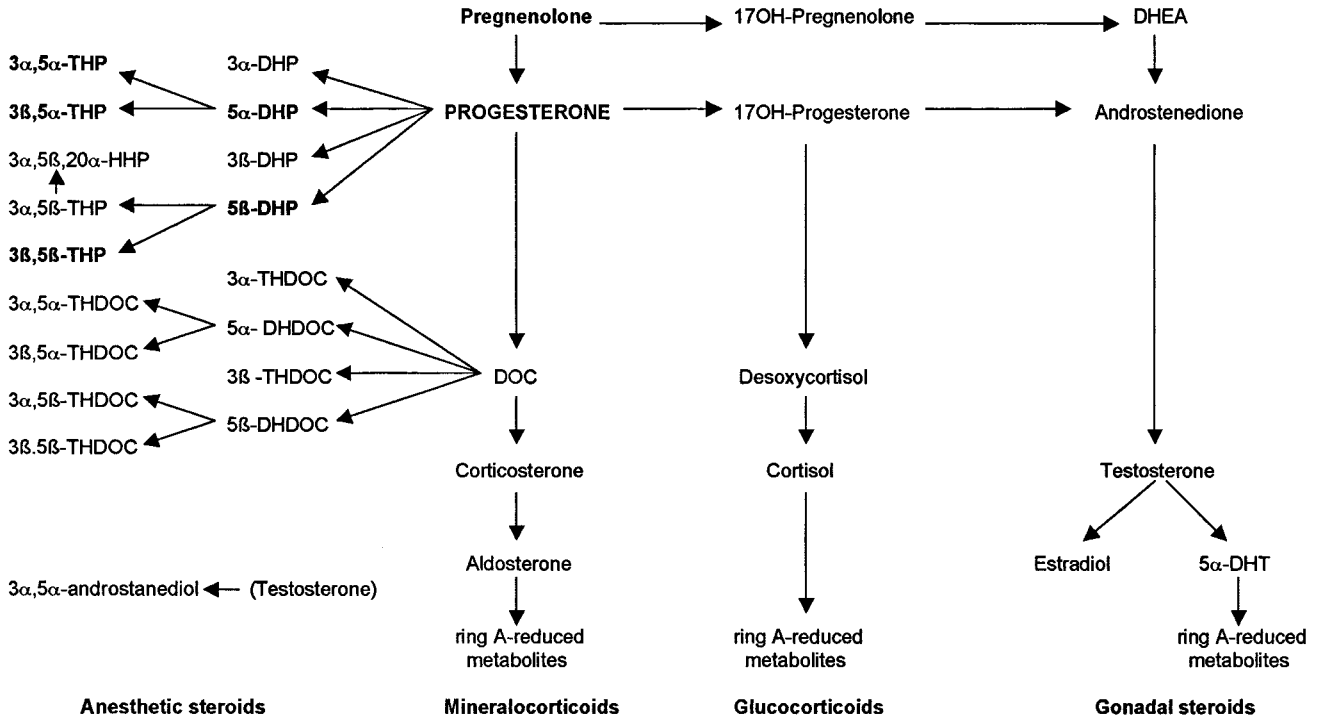

FIG. 4. Metabolism of pregnenolone. Steroids measured in this study are shown in *bold*. The list of anesthetic steroids is incomplete; some 19-nor derivatives and some androstane derivatives have also been shown to have anesthetic properties.

labor but were unable to relate this change to inhibition of uterine contractions (26).

Here we have shown that levels of 5α-DHP are elevated even more in previously healthy women who become significantly depressed for the first time during pregnancy. So far as we have been able to learn, there has been no previous investigation of this steroid in the depression of pregnant or nonpregnant subjects. During pregnancy, the prevalence of depression is 4–16% (27–32), whereas that postpartum is 10–28% (28, 33–35). In addition, about 1/4 suffer from the blues in the first 10 d following parturition (33), data similar to those obtained in this study.

No values for 5β-DHP were found in the literature. While both 5α-DHP and 5β-DHP given as a highly concentrated bolus produce anesthesia, 5β-DHP is considerably more potent, while 5α-DHP can cause convulsions (2). We showed that, at what were estimated to be physiological levels in rats, 5α-DHP increased motor activity, whereas 5β-DHP decreased it (36). Thus, these steroids, while differing only by the position of a single hydrogen, can have very different effects at low doses.

We were also unable to find any data at all for plasma levels of 3β,5β-THP.

Our levels of 3α,5α-THP (follicular 0.36 ± 0.24 nmol/liter rising to about 2.4 nmol/liter in the luteal phase) are in keeping with those found initially by Purdy *et al.* (37) using HPLC and RIA as here. More recent data by Genazzani *et al.* (38), omitting the HPLC step, gave higher mean follicular levels of 0.79 ± 0.30 SEM nmol/liter, rising 5-fold to 3.69 ± 0.96 SEM nmol/liter in the luteal phase. Recently, this group (Luisi *et al.*) (39), again omitting HPLC, reported levels in pregnancy that rose to about 50 ng/ml (158 nmol/liter) *i.e.* about 10 times higher than our values. We suspect that their values were influenced by a number of other cross-reacting steroids, including progesterone, because they did not validate their method for pregnancy, but relied entirely on cross-reactivity data (40, 41). This same group (42) recently found low levels of 3α,5α-THP in 18 women experiencing "blues" sampled on d 3 postpartum, compared with 22 controls (1.1 ± 0.5 *vs.* 2.3 ± 1.0 nmol/liter; $P \leq 0.001$). Our data were too scattered (over 2–7 d when levels were falling rapidly) to show any significant differences. However, we did find that the metabolite levels remained above nonpregnant levels during the early puerperium and 3α,5α-THP as well as 3β,5β-THP levels remained elevated even at 6 wk postpartum. Because 3α,5α-THP is anxiolytic, these higher levels may act to help women deal with the increased demands on time and effort in this usually stressful period.

We found only one paper in the literature in which 3β,5α-THP was measured in human plasma. Using gas chromatography/mass spectrometry, Romeo *et al.* (43) found levels of 3β,5α-THP of about 0.2 nmol/liter in 8 healthy male control subjects, values similar to those found by us (9). They found that the mean combined level in eight depressed male outpatients during a major unipolar depressive episode (0.6 nmol/liter) fell during fluoxetine treatment (to 0.2 nmol/liter, $P \leq 0.05$), whereas those of 3α,5α-THP rose from 2 to 5 nmol/liter ($P \leq 0.05$). A second study by the same group (43) showed the same trends but data for men and women were combined. No differences in progesterone levels were observed. Although 3α,5α-THP is clearly a positive allosteric modulator of the $GABA_A$ receptor, 3β,5α-THP may act as a functional antagonist.

The etiology of the depression occurring during preg-

nancy is still unclear. Our data strongly suggest that 3β,5α-THP may be involved. Buckwalter *et al.* (45), in a study of 15 women in the last month of pregnancy, found that higher levels of progesterone were associated with greater mood disturbances, and higher levels of DHEA with better mood. DHEA was not measured here, but the mean progesterone levels in the depressed groups were higher than those of the controls, but this difference failed to reach significance. O'Hara *et al.* (46) found lower levels of E2 in depressed subjects at wk 36 of gestation. Cognitive deficits (particularly deficit in verbal memory) observed by Buckwalter *et al.* (45) during pregnancy, in comparison to performance postpartum, were independent from the mood disturbances occurring during pregnancy.

The mood changes following parturition remain poorly understood. Blues have been associated with higher testosterone levels (45), lower DHEA levels (45), lower E2 levels (46), and reduced catecholamines (47), whereas postpartum depression has been attributed to low E2 levels (48).

According to the data obtained by Freeman *et al.* (49), levels of pregnanolones (combined 3α,5α-THP + 3α,5β-THP) above 96 nmol/liter are associated with fatigue, delayed verbal recall, and symbol copying. Thus the levels of 3α,5α-THP obtained by Luisi *et al.* (158 nmol/liter) (38) in late pregnancy would be expected to be associated with symptoms. Because only 3α,5α–THP was measured, and high levels of 3α,5β-THP as well as the other anesthetic metabolites of progesterone which may have similar effects, including 5β-DHP as found here, would also be expected to be elevated in pregnancy, these would be levels which, if correct, would be expected to cause serious alterations of functioning in virtually all pregnant women near term. We think it more likely that the lower amounts of the steroids measured here, when combined together with the other neuroactive progesterone metabolites, including those of desoxycorticosterone that also rises in pregnancy (16) and gives rise to NARS, do affect cognition and mood to the usually minor extent seen in many pregnant women, and occasionally to a disabling extent. These relatively high levels may act to provide some analgesia during the pain of labor and contribute to the tendency of most women to forget its intensity once it is over.

Acknowledgments

Received October 26, 2000. Accepted August 27, 2001.

Address all correspondence and requests for reprints to: Beverley E. Pearson Murphy, M.D., Ph.D., Psychoendocrinology Unit, 1033 Pine Avenue, West Montréal, Québec, Canada H3A 1A1. E-mail: bev.murphy@mcgill.ca.

This work was supported in part by the Fonds de Recherches Scientifiques du Québec, the Stairs Memorial Foundation, and the National Alliance for Research in Depression and Schizophrenia (NARSAD).

References

1. **Selye H** 1942 Correlations between the chemical structures and the pharmacological actions of the steroids. Endocrinology 30: 437–453
2. **Gyermek L, Soyka LF** 1975 Steroid anaesthetics. Anaesthesiol 42:331–344
3. **Figdor SK, Kodet MJ, Bloom BM, Agnello EJ, P'An SY, Laubach GD** 1957 Central activity and structure in a series of water-soluble steroids. J Pharmacol Exp Ther 119:299–309
4. **Holzbauer MK** 1974 Physiological aspects of steroids with anaesthetic properties. Med Biol 54: 227–243
5. **Paul SM, Purdy RH** 1992 Neuroactive steroids. FASEB J 6:2311–2322
6. **Mellon SH** 1994 Neurosteroids: biochemistry, modes of action, and clinical relevance. J Clin Endocrinol Metab 78:1003–1008
7. **Prince RJ, Simmonds MA** 1992 5β-Pregnan-3β-ol-20-one, a specific antagonist at the neurosteroid site of the $GABA_A$. Neurosci Lett 135:273–275
8. **Yu R, Ticku MK** 1995 Chronic neurosteroid treatment produces functional heterologous uncoupling at the gamma-aminobutyric acid A receptor complex in mammalian cortical neurons. Mol Pharmacol 47:603–610
9. **Murphy BEP, Allison CM** 2000 Determination of progesterone and some of its ring A-reduced metabolites in human serum. J Steroid Biochem Mol Biol 74:137–142
10. **Murphy BEP, Steinberg SI, Allison C** 2000 Determination of some neuroactive progesterone metabolites in pregnancy. Program of the Annual Meeting of The Endocrine Society, Toronto, Canada, 2000, p 558 (Abstract 2312)
11. **Spitzer RL, Williams JB, Kroenke K, Linzer M, deGruy FV, Hahn SR, Brody D, Johnson JG** 1994 Utility of a new procedure for diagnosing mental disorders in primary care. The PRIME-MDD Study. JAMA 272:1749–1756
12. **American Psychiatric Association** 1987 Diagnostic and statistical manual of mental disorders. Ed. 3, revised. Washington, D.C.: American Psychiatric Association
13. **Hamilton M** 1960 A rating scale for depression. J Neurol Neurosurg Psychiat 23:55–62
14. **Kennerley H, Gath D** 1986 Maternity blues reassessed. Psychiatr Dep 4:1–17
15. **Derogatis LR, Rickels K, Rock AF** 1976 The SCL-90 and the MMPI: A step in the validation of a new self-report scale. Br J Psychiatry 128:280–289
16. **Nolten WE, Lindheimer MD, Oparil S, Rueckert PA, Ehrlich EN** 1979 Desoxycorticosterone in normal pregnancy. II. Cortisol-dependent fluctuations in free plasma desoxycorticosterone. Am J Obstet Gynecol 133:644–648
17. **Tulchinsky D, Hobel CJ, Yeager E, Marshall JR** 1972 Plasma estrone, estradiol, estriol, progesterone and 17-hydroxyprogesterone in human pregnancy—normal pregnancy. Am J Obstet Gynecol 112:1095–1100
18. **Leb CR, Hu F-Y, Murphy BEP** 1997 Metabolism of progesterone by human lymphocytes: production of neuroactive steroids. J Clin Endocrinol Metab 82:4064–4068
19. **Hochberg R, Bandy L, Ponticorvo L, Welch M, Lieberman S** 1979 Naturally occurring lipoidal derivatives of 3β-hydroxy-5-pregnen-20-one; 3β,17α-dihydroxy-5-pregnen-20-one and 3β-hydroxy-5-androsten-17-one. J Steroid Biochem 11:1333–1340
20. **Albert DH, Prasad VV, Lieberman S** 1982 The conversion of progesterone into 5a-pregane-3,20-dione, 3-hydroxy-5-pregnan-20-one, and its fatty acid esters by preparations of bovine corpora lutea. Endocrinology 111: 17–23
21. **Poulin R, Poirier D, Thieriault C, Couture K, Belanger A, Labrie F** 1990 Wide spectrum of steroids serving as substrates for the formation of lipoidal derivatives in ZR-75-I human breast cancer cells. J Steroid Biochem 15:237–247
22. **Larner NJ, MacLusky JM, Hochberg RB** 1985 The naturally occurring C-17 fatty acid esters of estradiol are long-acting estrogens. J Steroid Biochem 22:407–413
23. **Parker Jr CR, Everett RB, Quirk Jr JG, Whalley PJ, Gant NF** 1979 Hormone production during pregnancy in the primigravid patient. Am J Obstet Gynecol 135:778–782
24. **Dombroski RA, Casey ML, MacDonald PC** 1993 The metabolic disposition of plasma 5α-dihydroprogesterone (5α-pregnane-3,20-dione) in women and men. J Clin Endocrinol Metab 77:944–948
25. **Löfgren M, Backström T** 1990 Serum concentrations of progesterone and α-dihydroprogesterone during labor and early postpartum. Acta Obstet Gynecol Scand 69:123–126
26. **Löfgren M, Holst J, Backström T** 1992 Effects *in vitro* of progesterone and two 5α-reduced progestins, 5α-pregnane-3,20-dione and 5α-pregnane-3α-ol-20-one, on contracting human myometrium at term. Acta Obstet Gynecol Scand 71:28–33
27. **Cox JL, Connor Y, Kendell RE** 1982 Prospective study of the psychiatric disorders of childbirth. Br J Psychiatry 140:111–117
28. **Gotlib IH, Whiffen VE, Mount H, Milne K, Cordy NI** 1989: Prevalence rates and demographic characteristics associated with depression in pregnancy and the postpartum. J Consult Clin Psychol 57:269–274
29. **Kumar R, Robson KM** 1984 A prospective study of emotional disorders in childbearing women. R J Psychiatr 144:35–47
30. **O'Hara MW, Neunaber DJ, Zekoski EM** 1984: A prospective study in postpartum depression: prevalence, course, and predictive factors. J Abnorm Psychol 93:58–171
31. **Watson JP, Elliott SA, Rugg AJ, Brough DI** 1984 Psychiatric disorder in pregnancy and the first postnatal year. Br J Psychiatry 144:453–462
32. **Kitimura T, Shima S, Sugawara M, Toda MA** 1993 Psychological and social correlates of the onset of affective disorders among pregnant women. Psychol Med 23:957–975
33. **O'Hara MW, Zekoski EM, Philipps LH, Wright EJ** 1990 Controlled prospective study of postpartum mood disorders: comparison of childbearing and nonchildbearing women. J Abnorm Psychol 99:3–15
34. **Boyce P, Parker G, Barnett B, Cooney M, Smith F** 1991 Personality as a vulnerability factor to depression. Br J Psychiatry 159:106–114
35. **Cox JL, Murray D, Chapman G** 1993 A controlled study of the onset, duration and prevalence of postnatal depression. Br J Psychiatry 163:27–31
36. **Dhar V, Stark R, Kraulis I, Murphy BEP** 1987 Contrasting effects of 5α- and

5β-pregnane-3,20-dione on the motor activity of ovariectomized rats. J Steroid Biochem 26: 577–580
37. **Purdy RH, Moore Jr PH, Rao PN, Hagino N, Yamaguchi T, Schmidt P, Rubinow DR, Morrow AL, Paul SM** 1990 Radioimmunoassay of 3α-hydroxy-5α-pregnan-20-one in rat and human plasma. Steroids 55:290–296
38. **Genazzani R, Petraglia F, Bernardi F, Casarosa E, Salvestroni C, Tonetti A, Napi RE, Luisi S, Palumbo MK, Purdy RH, Luisi M** 1998 Circulating levels of allopregnanolone in humans: gender, age, and endocrine influences. J Clin Endocrinol Metab 83: 2099–2103
39. **Luisi S, Petraglia F, Benedetto C, Nappi RE, Bernardi F, Fadalti M, Reis FM, Luisi M, Genazzani AR** 2000 J Clin Endocrinol Metab 85:2429–2433
40. **Murphy BEP** 2001 Allopregnanolone assays. Letter to the editor. J Clin Endocrinol Metab 86:1425
41. **Petraglia F** 2001 Allopregnanolone assays. J Clin Endocrinol Metab 86:1425
42. **Nappi RE, Petragilia F, Luisi S, Polatti F, Farina C, Genazzani AR** 2001 Serum allopregnanolone in women with postpartum "blues." Obstet Gynecol 7997: 77–80
43. **Romeo E, Ströhle A, Spalletta G, di Michele F, Hermann B, Holsboer F, Pasini A, Rupprecht R** 1998 Effects of antidepressant treatment on neuroactive steroids in major depression. Am J Psychiatry 155:910–913
44. **Ströhle A, Romeo E, Hermann B, Pasini A, Spalletta G, di Michele F, Holsboer F, Rupprecht R** 1999 Concentrations of 3α-reduced neuroactive steroids and their precursors in plasma of patients with major depression and after clinical recovery. Biol Psychiatry 45:27j4–277
45. **Buckwalter J, Stanczyk FZ, McCleary CA, Bluestein BW, Buckwalter DK, Rankin KP, Chang L, Goodwin TM** 1999: Pregnancy, the postpartum, and steroid hormones: effects on cognition and mood. Psychoneuroendcrinology 24:69–84
46. **O'Hara MW, Schlechte JA, Lewis DA, Wright EJ** 1991 Prospective study of postpartum blues. Biologic and psychosocial factors. Arch Gen Psychiatry 48:801–806
47. **Kuevi V, Causon R, Dixson AF, Everard DM, Hall JM, Hole D, Whitehead SA, Wilson CA, Wise JCM** 1983 Plasma amine and hormone changes in 'post-partum blues.' Clin Endocrinol 19:39–46
48. **Gregoire AJ, Kumar R, Everitt B, Henderson AF, Studd JW** 1996 Transdermal oestrogen for treatment of severe postnatal depression. Lancet 347:930–933
49. **Freeman EW, Purdy RH, Coutifaris C, Rickels K, Paul SM** 1993 Anxiolytic metabolites of progesterone: correlation with mood and performance measures following oral progesterne administration to healthy female volunteers. Neuroendocrinology 58:478–484
