BASIC SCIENCE: OBSTETRICS

Effect of endogenous steroid hormones on 17-alpha-hydroxyprogesterone caproate metabolism

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OBJECTIVE: Plasma concentrations of 17-alpha-hydroxyprogesterone caproate (17-OHPC) vary substantially in pregnant patients who receive an identical dose. Endogenous steroid hormones may alter 17-OHPC metabolism, which contributes to this large variability.

STUDY DESIGN: Pooled human liver microsomes were incubated with 17-OHPC alone or in combination with progesterone, hydroxyprogesterone, estrone, estradiol, or estriol. High-performance liquid chromatography with ultraviolet detection was used to quantify 17-OHPC.

RESULTS: Under the conditions that were studied, 17-OHPC metabolism was inhibited by 37% by a combination of endogenous steroid hormones. Progesterone alone significantly inhibited 17-OHPC metabolism by 28% ($P < .001$).

CONCLUSION: 17-OHPC metabolism is inhibited significantly by endogenous steroids and, in particular, progesterone. This effect may account for some of the large variation in plasma 17-OHPC concentrations that is seen in pregnant patients who receive a fixed dose of medication.

Key words: 17-alpha-hydroxyprogesterone caproate, drug-drug interaction, human liver microsome, metabolism, steroid hormone

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A synthetic progestin, 17-alpha-hydroxyprogesterone caproate (17-OHPC), is prescribed commonly for the prevention of recurrent, spontaneous preterm birth. The largest trial to investigate the clinical use of 17-OHPC for the reduction of recurrent, spontaneous preterm birth showed a 34% reduction in the incidence of recurrent preterm birth.[1] However, two-thirds of patients who were treated with the standard dose of 17-OHPC in this trial still delivered preterm.[1] Plasma concentrations of 17-OHPC vary substantially in pregnant women who are treated with the standard dose of 17-OHPC.[2,3] Because both 17-OHPC and endogenous steroid hormones are metabolized by the cytochrome P450 3A4 enzyme (CYP3A4), the variability in plasma concentration may, in part, be due to a drug-endogenous steroid interaction at the level of metabolism by CYP3A4.[3-10] Any alteration in 17-OHPC metabolism may affect plasma concentrations and ultimately impact the efficacy of 17-OHPC. Thus, the objective of this study was to examine the effect of endogenous steroid hormones on 17-OHPC metabolism in vitro with the use of human liver microsomes (HLM) that expressed the CYP3A4 enzyme. This study primarily evaluated the loss of 17-OHPC in the presence and absence of various concentrations of several steroid hormones.

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Materials and Methods

Chemicals

The 17-OHPC was purchased from the United States Pharmacopeia (Rockville, MD). Progesterone, 17-hydroxyprogesterone, estrone, 17-beta-estradiol, estriol, testosterone, 6β-hydroxytestosterone, and β-nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma-Aldrich (St. Louis, MO). All of the substrates and hormones were dissolved in dimethyl sulfoxide.

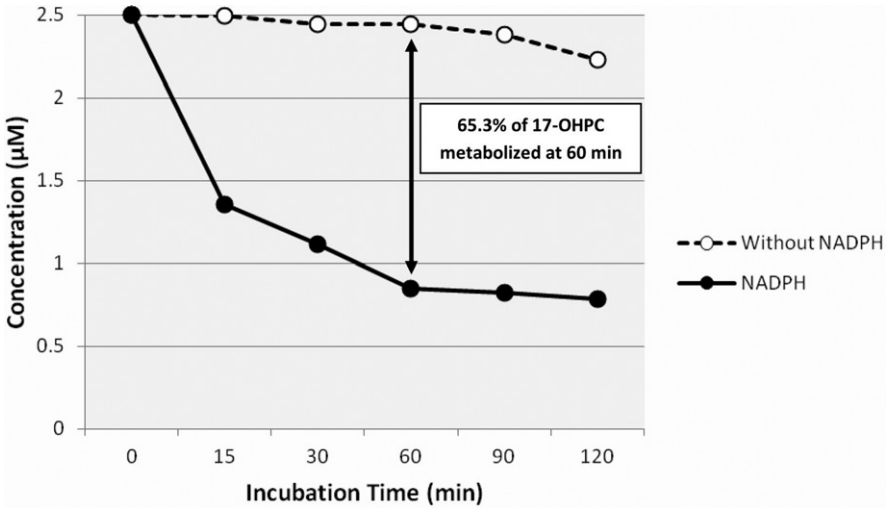
HLMs

Human liver samples were provided by the laboratory of Steve Strom, PhD, University of Pittsburgh, as part of the Liver Tissue and Cell Distribution System, #N01-DK-7-0004/HHSN267200700004C, under protocol 0411122. Tissue was obtained with informed consent and consent for research from organ donor material that was not suitable for transplantation or from patients who underwent liver resections mainly for cancer treatment. Tissue is provided to Liver Tissue and Cell Distribution System investigators without identifiers. This study was reviewed by the institutional review board at the University of Pittsburgh and was deemed exempt from full board review (PR011120226).

Preparation of HLMs

HLMs were prepared according to methods previously described.[3] Total protein concentration (20 mg/mL) was deter-

FIGURE 1
Time course of 17-OHPC metabolism by CYP3A4 in human liver microsomes



Displayed are the results from the preliminary experiments that were conducted to define the optimal incubation conditions. In the absence of β-nicotinamide adenine dinucleotide phosphate (NADPH; *dashed line*), essentially no 17-OHPC metabolism occurs, whereas metabolism occurs in the presence of NADPH (*solid line*). Overall, at 60 minutes, 65.4% of 17-OHPC was metabolized in the presence of NADPH. These findings support the findings that 17-OHPC is being metabolized by CYP3A4 in the human liver microsome system because NADPH is the cofactor that is necessary to activate the CYP3A4 enzyme. The *dashed* line also demonstrates that we had good recovery of 17-OHPC from the incubation tubes.

17-OHPC, 17-alpha-hydroxyprogesterone caproate; *CYP3A4*, cytochrome P450 3A4.

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mined by Lowry's method with bovine serum albumin as a standard. Pooled HLMs from 3 liver preparations were used in studies. CYP3A4 activity in pooled HLMs was characterized by quantification of the conversion of testosterone to 6β-hydroxytestosterone.[11]

Microsomal incubations

Incubation mixtures contained phosphate buffer (50 mmol/L; pH 7.4), magnesium chloride (1 mmol/L), and pooled HLM (0.05 mg of protein/mL, optimal protein concentration). Incubations were initiated by the addition of NADPH (1 mmol/L) and were carried out in a final volume of 250 μL in a 37°C shaking water bath. Dimethyl sulfoxide was used as a solvent for 17-OHPC and did not exceed 1% of the final reaction volume in all cases. Preliminary studies were conducted to establish the optimal protein concentration of HLM (0-2 mg/mL), incubation time (0-120 min), and 17-OHPC concentration (0-328 μg/mL) to be used in the experimental incubations. Depletion of parent compound in HLM was linear with respect to incubation time and microsomal protein concentration over ranges that were relevant to this study. From the data that used various concentrations of 17-OHPC, we also calculated the Vm (maximum rate of metabolism) and Km (substrate concentration at which the metabolic velocity is half of Vm) for 17-OHPC. Concentrations of 17-OHPC that are below the Km value were selected for the evaluation of the interaction between various hormones and 17-OHPC. The metabolic reactions were stopped by the addition of 250 μL of methanol. All samples were processed in triplicate. The mixture was centrifuged, and 100 μL of the supernatant was injected onto high-performance liquid chromatography for the analysis of 17-OHPC. Control incubations with no cofactor, no protein, and/or no substrate were performed concurrently to validate CYP3A4-dependent metabolism of 17-OHPC.

Steroid hormone-17-OHPC interaction

To evaluate the effect of various steroid hormones on 17-OHPC metabolism, we used fixed concentrations of 17-OHPC (2.5 μg/mL) that were incubated in the presence of a combination of steroid hormones (progesterone, 27.5 μg/mL; 17-hydroxyprogesterone, 1.5 μg/mL; estrone, 2 μg/mL; 17-beta-estradiol, 3.5 μg/mL; estriol, 11 μg/mL) or with individual steroid hormones that included progesterone, 17-hydroxyprogesterone, estrone, 17-beta-estradiol, and estriol at the same concentrations. Both the concentration of 17-OHPC and the endogenous steroid hormones that were used for this experiment were much higher than the concentrations that were observed during pregnancy (micrograms per milliliter vs nanograms per milliliter). These higher concentrations were necessary to ensure adequate substrate for the detection of metabolic interactions over a prolonged period of incubation in the HLM. To more closely mimic in vivo conditions, the ratio of 17-OHPC to endogenous steroid hormone concentration was kept physiologically proportional to the concentrations that were reported during pregnancy. Once significant interactions were identified with the use of the supraphysiologic concentrations of various hormones and 17-OHPC, we performed additional experiments with physiologic concentrations of progesterone and 17-OHPC. Specifically, for these studies in which physiologic concentrations were used, incubations were prepared as described earlier but with a final HLM concentration of 0.0025 mg of protein/mL, 17-OHPC concentration of 50 ng/mL, and progesterone concentrations of 183 and 550 ng/mL. All incubations were carried out for 60 minutes and were performed in duplicate. Reactions were stopped and processed as described earlier.

Additionally, we evaluated whether there is a concentration-dependent relationship between progesterone and the degree of inhibition of 17-OHPC. Various concentrations of progesterone

(0 μg/mL, 1.72 μg/mL, 3.44 μg/mL, 6.88 μg/mL, 13.75 μg/mL, 27.5 μg/mL, 55 μg/mL, 110 μg/mL) were incubated with 17-OHPC (2.5 μg/mL). The incubation solution was otherwise prepared as described earlier. All incubations were carried out for 60 minutes and were performed in triplicate. Reactions were stopped and processed as described earlier.

High-performance liquid chromatography–ultraviolet analysis for 17-OHPC

The high-performance liquid chromatography system comprised a Waters 2695 Separations Module attached to Waters 2998 Photodiode Array Detector (Waters Corp, Millford, MA). Chromatographic separations were achieved on a 250 ×4.6 mm, 5 μm Waters Symmetry C18 column (Interlink Scientific Services Limited, Dartford, UK) with an isocratic elution that was monitored at 242 nm with a mobile phase (delivered at 1.0 mL/min) of 90% (vol/vol) methanol in water. The retention time of 17-OHPC was 5.9 minutes. The concentration of 17-OHPC in samples was quantitated according to its calibration curve. The intraday and interday variations that were expressed as coefficient of variation did not exceed 10% in any of the assays. The limit of detection was 5 ng, and the limit of quantitation was 20 ng on the column.

Data analysis

Data from the 60-minute incubation time point was analyzed by 1-way analysis of variance with a Dunnett's correction for multiple comparisons with a control. Statistical analysis was performed with Stata software (version 11; StataCorp, College Station, TX).

RESULTS

Incubation conditions for HLMs

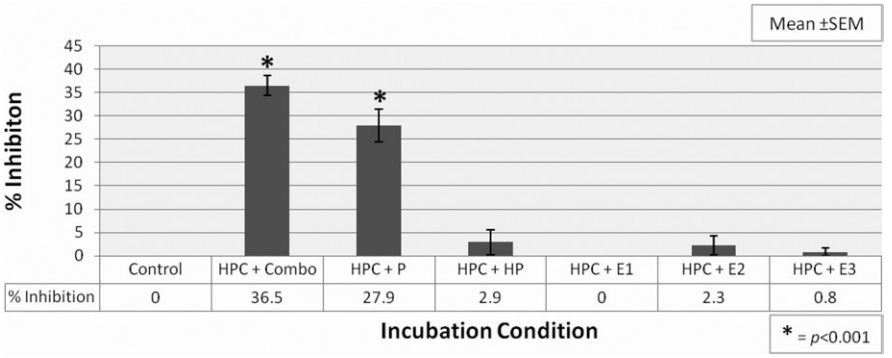
Preliminary experiments were carried out to establish the optimal incubation conditions for the HLM system. Incubations were carried out both in the presence and absence of NADPH, which was the cofactor necessary for CYP3A4-mediated metabolism. Figure 1 shows that, in the absence of NADPH, 17-OHPC was not metabolized. With the addition of NADPH, the concentration of 17-OHPC decreased by 65.3%, which indicated robust metabolism within 60 minutes. This finding supports the fact that 17-OHPC was metabolized by cytochrome P450 enzymes in the HLM system. Additionally, in the absence of NADPH, there was consistent recovery of 17-OHPC from the incubation tubes, which indicated that the decrease that was observed in the presence of NADPH was secondary to metabolism and not spontaneous decomposition. Beyond 60 minutes, little metabolism occurred that likely was due to exhaustion of the NADPH. Thus, for the purposes of this experiment, 60 minutes was the time point that was selected for all data analysis.

Effects of endogenous steroid hormones on 17-OHPC metabolism

To investigate the effects of endogenous steroid hormones on 17-OHPC metabolism that was catalyzed by CYP3A4 in HLM, 17-OHPC was added to reaction mixtures with individual steroids or a combination of steroids. Approximately 59% of 17-OHPC was metabolized under control conditions with NADPH. When 17-OHPC was incubated with a combination of the endogenous steroid hormones, only 37.1% of 17-OHPC was metabolized, which was significantly less compared with control conditions ($P < .001$). Also, in the presence of progesterone alone, only 42.1% of 17-OHPC was metabolized, which was significantly less when compared with control ($P < .001$). Rates of 17-OHPC metabolism that were similar to control conditions were observed when 17-OHPC was incubated with 17-alpha-hydroxyprogesterone (58.4%), estrone (62.1%), estradiol (60.8%), and estriol (60.9%).

The same dataset was used to calculate the percent inhibition of 17-OHPC metabolism. Percent inhibition was calculated by subtraction of the percent metabolism in the presence of steroid hormone from the percent metabolism under control conditions (absence of steroid hormone). This difference was then divided by the percent metabolism under control conditions (Figure 2). Control conditions were considered to be zero inhibition and were used as the comparison for the other incubation conditions. When incubated with a combination of steroid hormones, 17-OHPC metabolism was significantly inhibited by 36.5% ($P < .001$) relative to control conditions. Also, in the presence of pro-

FIGURE 2
Inhibition of 17-OHPC metabolism



Displayed are the incubation conditions along the x-axis and the percent inhibition of 17-OHPC metabolism along the y-axis. Percent inhibition was calculated by subtraction of the percent metabolism in the presence of steroid hormone from the percent metabolism under control conditions and division of the difference by the percent metabolism under control conditions. One-way analysis of variance with a Dunnett correction for multiple comparisons to a control was used to compare the results from each incubation category to control conditions.

17-OHPC, 17-alpha-hydroxyprogesterone caproate; *E1*, estrone; *E2*, 17-beta-estradiol; *E3*, estriol; *HP*, 17-hydroxyprogesterone; *HPC*, 17-alpha-hydroxyprogesterone caproate; *P*, progesterone.

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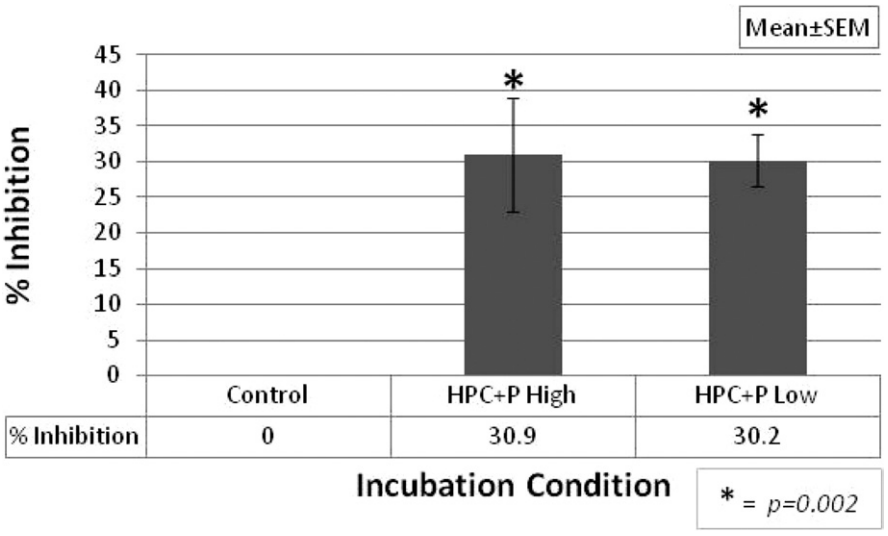
FIGURE 3
Inhibition of 17-OHPC metabolism at physiologic hormone levels



Displayed are the incubation conditions along the x-axis and the percent inhibition of 17-OHPC metabolism along the y-axis. Progesterone at high and low physiologic concentrations significantly ($P = .002$) inhibited the metabolism of 17-OHPC. One-way analysis of variance with a Dunnett correction for multiple comparisons to a control was used to compare the results from each incubation category to control conditions.

17-OHPC, 17-alpha-hydroxyprogesterone caproate; *HPC*, 17-alpha-hydroxyprogesterone caproate; *P*, progesterone.

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gesterone, 17-OHPC metabolism was inhibited by 27.9%, which was significant when compared with control conditions ($P < .001$). Relative to the control, the presence of 17-hydroxyprogesterone (2.9%), estrone (0%), 17-beta-estradiol (2.3%), or estriol (0.8%) did not result in significant inhibition of 17-OHPC metabolism.

Effect of physiologic concentrations of progesterone on 17-OHPC metabolism

The impact of physiologic concentrations of progesterone on 17-OHPC metabolism is shown in Figure 3. In the presence of both high (550 ng/mL) and low (183 ng/mL) concentrations of progesterone, 17-OHPC metabolism was inhibited by 30.9% and 30.2%, respectively. This inhibition was significant ($P = .002$) compared with control conditions and similar to the degree of inhibition that is seen with the supraphysiologic concentrations of 17-OHPC and progesterone that are seen in Figure 2.

Figure 4 shows a significant association ($R^2 = 0.86$; $P = .01$) between increasing progesterone concentrations and the degree of inhibition of 17-OHPC metabolism. To make these findings clinically relevant, the ratio of progesterone to 17-OHPC at each concentration is displayed above each data point in Figure 4. Physiologic ratios of progesterone concentrations to 17-OHPC concentrations during normal pregnancy range from 2.5:1 to 60:1. The study presented here used pooled microsomes from 3 different livers to determine the potential of an interaction between various hormones and 17-OHPC. The study was not designed to address the variability in the magnitude of the interaction between different subjects. Variation in the Km values of endogenous hormones will contribute to any observed variability in the magnitude of inhibition among different subjects.

COMMENT

Plasma concentrations of 17-OHPC are highly variable in pregnant women. This variability may be due to variability in the absorption, distribution, and metabolism of 17-OHPC. 17-OHPC has been shown to be metabolized by enzymes in the liver and placenta. Large variability in the plasma concentrations of 17-OHPC may be due to variability in hepatic and placental metabolism in addition to variability in metabolism at other sites.

In this study, we have shown that progesterone inhibits the metabolism of 17-OHPC in an in vitro system of HLM that was prepared from human livers. These findings are biologically plausible because progesterone and 17-OHPC are both metabolized by CYP3A4.[3-10] We found that incubation with a combination of all the steroids or with progesterone alone resulted in significant inhibition of 17-OHPC metabolism by 36.5% and 27.9%, respectively (Figure 2). Individual incubations with the other steroid hormones had little to no effect on 17-OHPC metabolism. Thus, the inhibition noted in the presence of the combination of steroid hormones is likely driven by progesterone.

When HLM systems are used to investigate drug-drug interactions, higher concentrations of drugs/hormones are generally used. Adequate substrate is required in the incubation mixture not only to detect the depletion of the parent drug of interest but also to quantify metabolite formation. The studies we performed identified progesterone as the steroid with the greatest impact on 17-OHPC metabolism. We then used physiologic concentrations of 17-OHPC and progesterone to further validate our findings. We found significant inhibition ($P = .002$) of 17-OHPC metabolism over a wide range of physiologic concentrations that may be observed during gestation.

Additionally, we were able to demonstrate a positive relationship between progesterone concentration and the degree of inhibition of 17-OHPC metabolism (Figure 4). The progesterone concentration–dependent inhibition of 17-OHPC metabolism would support the notion that variable progesterone concentrations that are observed during pregnancy may contribute to the wide variability in 17-OHPC concentration that is seen in patients who are treated with a constant, fixed dose of the medi-

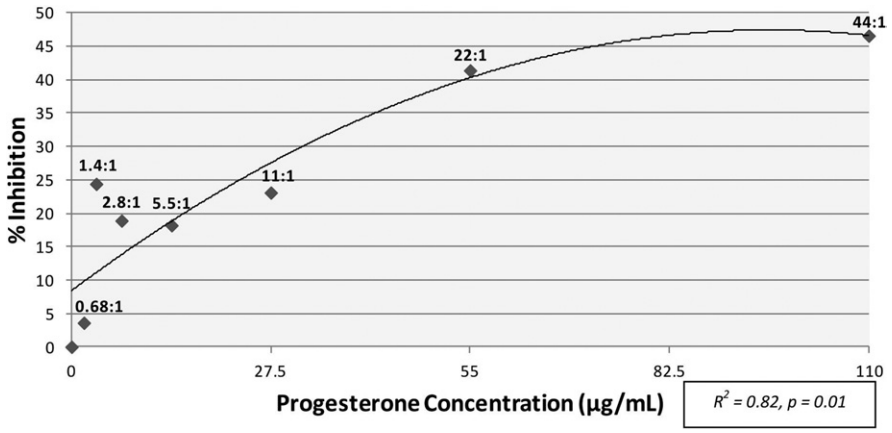
cation. This observation suggests that plasma 17-OHPC concentrations may be affected by circumstances that affect plasma progesterone concentrations, such as gestational age and/or placental number. In singleton gestations, the plasma concentration of progesterone increases from 20-150 ng/mL during the second trimester to 100-400 ng/mL at term.[12-14] In twin gestations, progesterone levels are approximately 60% higher throughout gestation compared with singleton gestations.[15] On average, progesterone increases from 124-230 ng/mL in the second trimester to 220-550 ng/mL in the third trimester in twin gestations.[15-17] Thus, because progesterone may inhibit 17-OHPC metabolism, an increase in 17-OHPC concentration would be expected as pregnancy progresses. Indeed, among both singleton and twin gestations, plasma 17-OHPC concentrations increase as gestation advances; however, it is unclear whether this observation is due to the increased inhibition of CYP3A4, to the increased release of 17-OHPC from maternal fat stores, or to an increased number of castor oil depots.[2]

There were some limitations to this study. First, it was an in vitro study, and the absence of other metabolic enzymes may result in an incomplete metabolic profile of 17-OHPC. However, previous studies have demonstrated that 17-OHPC is metabolized primarily by CYP3A4.[3] Thus, the absence of other metabolic enzymes probably would not affect the results of this study significantly. Additionally, because microsomes lack genetic material, we could not study the induction of the CYP3A4 enzyme, which could also affect the overall metabolic profile.

In addition to the 17-OHPC/endogenous steroid interaction that was observed, both body mass index and race have been shown to impact the concentration of 17-OHPC in patients with a twin gestation who received a standard dose of the medication.[2] Also, several commonly prescribed medications in pregnancy are also substrates, inhibitors, and/or inducers of CYP3A4.[18-21] These factors may also contribute to the large interindividual variability that was observed in 17-OHPC plasma concentrations. Clinically, a better understanding of these interactions is important because alterations in 17-OHPC metabolism can affect overall drug concentration and possibly impact the efficacy of 17-OHPC in the prevention of preterm birth. As more is learned about 17-OHPC, it may eventually be necessary to adjust doses of the medication to target a therapeutic level, especially in patients with lower or higher progesterone levels or who take medications that are also CYP3A4 substrates, inhibitors, and/or inducers. The overall magnitude of inhibition of 17-OHPC in a given subject is expected to depend of multiple factors that include the concentration of progesterone and 17-OHPC at the sites of metabolism and the relative affinity of these compounds for their metabolic enzymes, among others.

In conclusion, endogenous steroid hormones appear to cause a marked inhibition of 17-OHPC metabolism in vitro. Specifically, a combination of hormones and/or progesterone alone resulted in the most significant inhibition. We were able to demonstrate consistent inhibition of 17-OHPC metabolism at physiologic concentrations of 17-OHPC and progesterone. Additionally, we showed a significant association between progesterone concentration and the degree of inhibition of 17-OHPC metabolism. These findings support the notion that interindividual variation of endogenous steroid hormones throughout gestation contributes to the wide variability of 17-OHPC plasma concentrations that are observed in women who are treated with a fixed dose of medication. ■

FIGURE 4
Inhibition of 17-OHPC metabolism with increasing concentrations of progesterone



The progesterone concentration is displayed along the x-axis (range, 0–110 µg/mL), and the percent inhibition of 17-OHPC metabolism is displayed along the y-axis. There is a significant association between increasing concentrations of progesterone and the degree of inhibition of 17-OHPC metabolism (R^2 = 0.86; P = .01). In other words, as progesterone concentration increases, 17-OHPC metabolism decreases. Above each data point is the ratio of progesterone to 17-OHPC. Physiologic ratios range from 2.5:1 to 60:1.

17-OHPC, 17-alpha-hydroxyprogesterone caproate.

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