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Pharmacodynamics of Follicle Stimulating Hormone (FSH) in Postmenopausal Women during Pulsed Estrogen Therapy: Evidence That FSH Release and Synthesis Are Controlled by Distinct Pathways

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17β-Estradiol (E2) exerts negative feedback effects at the hypothalamo-pituitary level on serum FSH. This study investigated the effects of repeated daily administration of intranasal E2 (S21400) on the pharmacokinetics (PK) of E2 and estrone (E1) and the pharmacodynamics (PD) of FSH and assessed the PK/PD relationship between E2 and FSH using population model-dependent analysis. Postmenopausal volunteers (n = 24) received according to a balanced cross-over design, two 28-d treatments separated by a 2-month wash-out period: 300 μg E2, either alone or combined with oral dydrogesterone (20 mg/d) during the last 14 d of one of the treatments. Absorption of E2 was rapid, with maximal plasma concentrations at 10–30 min, returning to postmenopausal levels within 12 h. Over the 24-h period, FSH levels showed a U curve, with a minimum around 8 h after E2 administration. Moreover, over the treatment period, FSH basal values decreased by 17% between d 1 and 14 and an additional 5% between d 14 and 28. A PK/PD model described these short- and mid-term effects, possibly reflecting separate regulation mechanisms by E2 on FSH release and biosynthesis, respectively. The administration of progestin had no influence on E1, E2, and FSH model parameters. This study suggests that daily transient tissue exposure to E2 after pulsed estrogen therapy elicits short- and mid-term effects on the gonadotropin axis. (*J Clin Endocrinol Metab* 88: 5405–5413, 2003)

FSH IS A heterodimeric hormone secreted from the pituitary. It is composed of an α-subunit that is common to LH, TSH, and human chorionic gonadotropin and a β-subunit that is unique. Two carbohydrate chains are linked to both α- and β-subunits. Glycosylation of the subunits is involved in the biological activity of the hormones, generating 20 circulating isoforms of FSH. Gonadotropin secretion is under the control of GnRH and gonadal peptides such as activins, inhibins, and gonadal steroids [progesterone and 17β-estradiol (E2)] (1). FSH is considered to be one of the main hormonal markers of menopausal status (2), as levels of the hormone are markedly increased at menopause. One year after menopause, FSH levels are 10- to 15-fold higher than those during the follicular phase in women of reproductive age (3–5). Estrogen replacement therapy has been shown to produce a dose-related decrease in serum FSH levels in postmenopausal women (6) after oral E2 administration (7) as well as after E2 administration by nonoral routes, such as transdermal, sublingual, and intravaginal estrogen (8–11). Although nyctemeral variations in FSH levels and during the menstrual cycle have been well characterized, specific data from postmenopausal women receiving hormone replacement therapy are limited.

Intranasal administration of an aqueous E2 solution (complexed with a methylated β-cyclodextrin) leads to a rapid, but short-lasting, rise in plasma E2 levels (800–1200 pg/ml). This rise is followed by a rapid distribution to the tissues and a return to levels observed in postmenopausal women within 8–12 h. Therefore, it introduces the concept of pulsed estrogen therapy (12, 13). Besides clinical advantages associated with the transient exposure compared with oral or transdermal routes (lower breast and endometrial stimulation) at doses providing the same efficacy on climacteric symptoms or preventing postmenopausal bone loss (14, 15), the pulsed kinetic profile of serum E2 provided by intranasal administration represents a unique model to study the dynamic effects of E2 on FSH levels in postmenopausal women.

The aim of the current study was to investigate the short- and mid-term effects of intranasal E2 on the time course of serum FSH levels assessed over a 24-h period on d 1, 14, and 28 of a 28-d treatment in postmenopausal women and to investigate the potential effect of progestin coadministration.

Subjects and Methods

Subjects

Twenty-four healthy, Caucasian, postmenopausal women volunteers, aged 57 ± 5 yr (mean ± SD), with a mean body weight of 63 ± 7 kg and a mean body mass index of 24 ± 2 kg/m^2 were included in this study. All subjects were nonsmokers and had been menopausal for at least 3 yr (mean, 8.2 ± 6.0 yr). The concomitant treatments, which were likely to change the metabolism of E2, alter the nature or development of mucosal secretions, or modify the hypothalamo-pituitary axis regulation, were not allowed. Women who had received treatment with estrogen and/or progestogen by the oral or systemic route during the

Abbreviations: E1, Estrone; E2, 17β-estradiol; h, human; PD, pharmacodynamics; PK, pharmacokinetics.

30 d preceding the selection visit were excluded of the study. Postmenopausal status was confirmed by FSH values greater than 30 mIU/ml and E2 values less than 20 pg/ml. All subjects gave their written, informed consent and underwent a complete medical examination before inclusion. The protocol of the trial was approved by the ethics committee of Versailles, France.

Study design

This open study was performed with 24 subjects receiving during 2 periods, 2 28-d treatments with daily administration of intranasal E2 (S21400). According to a balanced, randomized, cross-over design, a progestin was administered daily the last 14 d of 1 of the 2 treatment periods. The first (d 1–28) and second treatment periods (d 90–117) were separated by a 2-month wash-out (d 29–89) designed to avoid interferences between the 2 treatment periods. S21400 was administered intranasally in the morning as a single daily dose of 300 μg (1 spray/nostril) for 28 d. Each spray had a volume of 70 μl and delivered 150 μg E2. Oral progestin (20 mg/d dydrogesterone, Solvay Pharma, Suresnes, France) was taken within 10 min before or after the intranasal administration during the last 14 d of 1 of the 2 treatment periods.

Blood sampling

Blood was collected for FSH, E2, and estrone (E1) assays before intranasal drug administration at inclusion; on d 1, 14, and 28 of each treatment cycle; 15 and 30 min before E2 administration; and 10, 20, 30, and 45 min and 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h (immediately before the next dosing) after administration. During the wash-out period blood was collected for E1 and E2 assays only. All samples were obtained by venipuncture or via a forearm catheter, then collected into glass tubes without anticoagulant before centrifugation (3000 rpm for 10 min at room temperature). Serum samples were then aliquoted into polypropylene test tubes and stored at −20 C until analyzed.

Assays

E1 and E2 concentrations were quantified in duplicate serum samples by a method based on a modified RIA using commercial kits (Biomerieux, Craponne, France) in which a liquid/liquid extraction was followed by a chromatographic separation of the extracted steroids. An RIA was then performed, based on a competitive inhibition reaction between 4-fold [^{3}H]E1 or [^{3}H]E2 at a fixed concentration, with the steroid contained in standards or samples to be assayed, against anti-E1 or anti-E2 antibodies. The accuracy and precision of this assay have been validated to be less than 20% (12), and the limits of detection were 5 and 20 pg/ml for E2 and E1, respectively.

FSH concentrations were determined in duplicate using an immunoradiometric assay for follitropin in human serum (FSH IRMA kit, Biocode Biotechnology, Sclessin, Belgium). The limit of detection of this assay was 0.23 mIU/ml, and cross-reactivity with human chorionic gonadotropin, human (h) LH (hLH), hTSH, and hPRL was less than 0.001%. The intra- and interassay coefficients of variation assessed in the study using Lyphocheck Immunoassay Plus Controls (Bio-Rad Laboratories, Irvin, CA) were less than 4% and 10%, respectively. Six FSH standards were used and were calibrated against the WHO Second International Reference Preparation IRP 78/549.

Pharmacokinetic evaluation

A population model-dependent PK analysis was performed using NONMEM software version IV level 1.2 (University of California, San Francisco, CA), to obtain *post hoc* individual parameters. E1 and E2 concentration time data were fitted separately to three compartment models that were parameterized, without taking into account the absolute bioavailability (F), in terms of apparent clearance, where CL/F (CL, total clearance), Q2/F, and Q3/F (Q2 and Q3, intercompartmental clearance), and of apparent volumes of distribution, with Vc/F (Vc, central compartment volume), V2/F, and V3/F (V2 and V3, peripheral compartment volumes). The absorption of E2 and the formation of E1 were parameterized with regard to duration as a zero order process (T_{K0}). Basal levels of the endogenous hormones were taken into account in the modeling. The influence of covariates was statistically tested for the primary pharmacokinetic parameters (clearance, volumes of distribution, duration of the zero order process for absorption of E2 or formation of E1, and basal levels of endogenous estrogens) using the likelihood ratio test. The covariates tested were coadministration of progestin, single *vs.* repeated administration, first E2 dose (d 1 and 90) *vs.* repeated administrations (d 14, 28, 103 and 117), influence of the cycle (first *vs.* second cycle), and influence of the cycle day (d 1 and d 90 *vs.* d 14 and d 103 *vs.* d 28 and d 117).

Pharmacokinetic/pharmacodynamic evaluation

A mechanistic-based model was developed, using NONMEM software, to link the PK of E2 to the PD effects of FSH suppression. Two PK/PD submodels were necessary to describe the dual action of E2 on FSH. Model 1 (used to describe the inhibition of FSH secretion by E2 that takes place within several hours) was an indirect inhibitory sigmoid Emax model, which related plasma concentrations of E2 to FSH serum levels. Model 2 (used to describe the inhibition of FSH biosynthesis by E2 which takes place within several days) was a drug exposure response time model relating the E2 exposure to the predose values of FSH. The indirect inhibitory sigmoid Emax model (model 1) was set up according to the following equations:

$$\frac{dFSH}{dt} = K_{in} \times I(t) - K_{out} \times FSH \quad (1)$$

where K_{in} represents the zero order constant for production of FSH, I(t) is the inhibitory function, and K_{out} defines the first order rate constant for the loss of FSH.

$$I(t) = 1 - \frac{E\,max \times C_p^{\gamma}}{C_p^{\gamma} + IC_{50}^{\gamma}} \quad (2)$$

where Emax represents the maximum effect attributed to the drug (E2), C_p is the plasma concentration of E2, γ is the Hill factor, and IC_{50} represents the E2 concentration producing 50% of the maximum inhibition. Model 1 assumes that under baseline conditions with no drug administered:

$$K_{in} = K_{out} \times FSH_0 \quad (3)$$

The drug exposure response time model (model 2) was set up on the predose values of FSH (FSH_0), according to the following formula:

$$FSH_0 = FSH_{0\,initial} \times (1 - A \times AUC_{\tau} \times \exp(-K_{eq} \times time)) \quad (4)$$

where FSH_0 is the level of FSH before each administration, $FSH_{0\,initial}$ is the baseline value of FSH before the first E2 administration, $AUC\tau$ is the E2 exposure during a dosing interval (24 h), A is a constant that when multiplied by $AUC\tau$ gives the percent maximum decrease in FSH_0 from $FSH_{0\,initial}$, and K_{eq} is the rate constant of equilibration, defining the delay in the effect of E2 on the FSH biosynthesis.

Challenging the pharmacokinetic/pharmacodynamic model

To test the ability of the model to predict data coming from new studies, the approach suggested by Bruno *et al.* (16), based on quantitative evaluations (building data set) of model predictions on individual subject (validation dataset) FSH concentrations, was applied. Data were computed by Bayesian feedback. To challenge the short- and mid-term actions of E2 on FSH levels, two validation datasets from previously published studies (12, 13) were used: a single dose study involving three doses of S21400 (100, 300, and 450 μg) (12) and a dose-ranging study involving four doses of S21400 (100, 200, 300, and 400 μg), given once daily for 12 wk (13).

Statistical analysis

All statistical calculations were performed using SAS 6.12 software (SAS Institute, Inc., Cary, NC). Inter- and intrasubject variabilities of E1, E2, and FSH model-dependent parameters were calculated by comparing individual parameter values from each period of treatment. For the PK data, six time periods were considered, one for each day of PK evaluation (d 1, 14, 28, 90, 103, and 117). For the PD data, two periods

were considered, one for each cycle. Data were analyzed by variance analysis (ANOVA), which included both a subject effect and a period effect. The intersubject variability was based on the estimate of the subject effect. The error term of the ANOVA on untransformed values, which accounted for unexplained phenomena, was an estimate of the intrasubject variability. For all statistical analysis, $P < 0.05$ was considered significant.

Results

Pharmacokinetics

E2. The mean concentration of endogenous serum E2 in the postmenopausal women included in the study, measured 30 min before E2 administration, was 14 ± 7 pg/ml, and this concentration varied only slightly throughout the day, as assessed during the wash-out period (range, 10.4–14.5 pg/ml). After nasal administration of 300 μg E2 as S21400, the time course of observed serum E2 concentrations was characterized by relatively high levels of E2 of short duration (Fig. 1A). On d 1, the maximum mean E2 concentration observed was 1014 ± 505 pg/ml (mean ± SD), which was reached 10 min after administration and was followed by a rapid tissue distribution. Two and 4 h after administration, the E2 concentration observed represented only 13% (*i.e.* 132 pg/ml) and 6% (*i.e.* 61 pg/ml), respectively, of the maximum mean observed value. E2 concentrations returned to levels similar to those in untreated postmenopausal women by 12 h. The E2 PK profiles observed on d 1, 14, and 28 were superimposable (Fig. 1A). When model-dependent analysis was carried out, the E2 concentration-time data were best described by a three-compartment open model. Coadministration of progestogen was found to have no influence on the PK of E2, either on the days within a treatment period or on the treatment period itself.

The mean population values of the E2 primary PK parameters obtained with the model were as follows: CL/F, 207 liters/h; Vc/F, 178 liters; Q2/F, 130 liters/h; V2/F, 1100 liters; Q3/F, 161 liters/h; V3/F, 107 liters; duration of the zero order input (T_{K0}), 0.22 h; and endogenous baseline level of E2, 12 pg/ml (Fig. 1C). The residual error terms were estimated as 17% and 3.5 pg/ml for the multiplicative and the additive parts, respectively. The secondary PK parameters are reported in Table 1. The intra- and intersubject variabilities of the main PK parameters for E2 were less than 28%, with the exception of the interindividual variability of the predicted total serum concentration (C_{max}), which was 33% (Table 2).

E1. With regard to E1, the mean endogenous serum level in the postmenopausal women in this study 30 min before E2 administration was 12 ± 7 pg/ml (mean ± SD), and this level varied throughout the day (as assessed during the wash-out period) from 11 ± 6 to 14 ± 9 pg/ml, for the lowest and highest mean values, respectively.

A maximum mean E1 concentration of 317 ± 153 pg/ml (mean ± SD) was observed 20 min after the nasal administration of 300 μg E2 as S21400 (Fig. 1B). Two and 4 h after nasal administration, the E1 levels observed represented 33% (*i.e.* 105 pg/ml) and 23% (*i.e.* 44 pg/ml), respectively, of the maximum mean observed value after E2 treatment. Twenty-four hours after E2 administration, the E1 levels were slightly higher (33 ± 20 pg/ml) than the endogenous pretreatment levels. The E1 profiles observed on d 1, 14, and 28 were superimposable. When model-dependent analysis was carried out, the E1 concentration-time data were best described by a three-compartment open model. None of the tested covariates was found to influence the pharmacokinetics of E1.

The mean population values of the E1 primary PK parameters obtained with the final model were as follows: CL/F, 183 liters/h; Vc/F, 668 liters; Q2/F, 40 liter/h; V2/F, 2140 liters; Q3/F, 711 liter/h; V3/F, 866 liters; duration of the zero order input (T_{K0}), 0.30 h; and endogenous baseline level of E1, 13 pg/ml (Fig. 1C). The residual error terms were estimated to be 13% and 2.6 pg/ml for the multiplicative and additive parts, respectively. The mean ratios of E1 to E2 systemic exposure and the secondary PK parameters of in-

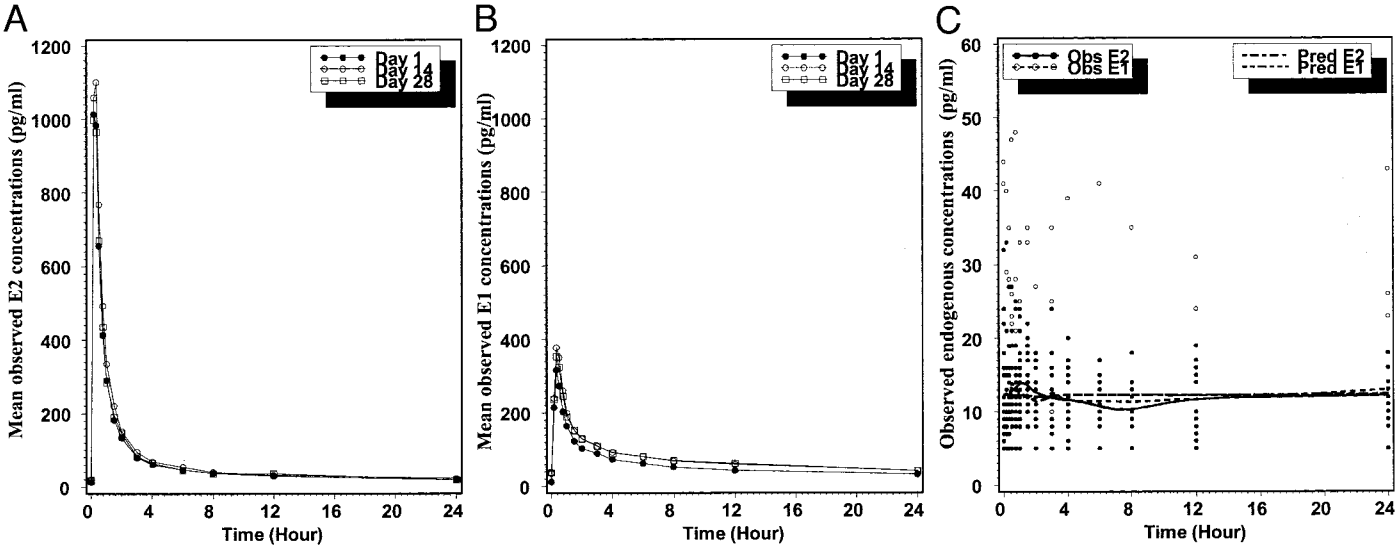

FIG. 1. Mean E2 and E1 pharmacokinetics. A, Mean observed serum E2 concentrations *vs.* time. B, Mean observed serum E1 concentrations *vs.* time. C, Observed endogenous E2 (●) and E1 (○) concentrations *vs.* time. The lines represent cubic spline through the observed and the individual model predictions, respectively.

TABLE 1. Model-dependent secondary pharmacokinetic parameters of E2 and E1

	First 28-d treatment period			WO	Second 28-d treatment period		
	d 1	d 14	d 28		d 90	d 103	d 117
E2 parameters							
C_{min} (pg/ml)	21 ± 6.8	19 ± 4.9	20 ± 4.3		20 ± 4.8	23 ± 7.5	21 ± 5.7
C_{max} (pg/ml)	1355 ± 495	1476 ± 544	1358 ± 519		1318 ± 533	1360 ± 560	1270 ± 477
AUCexo (pg/h·ml)	1511 ± 528				1458 ± 404		
AUC_{24}exo (pg/h·ml)	1328 ± 398	1541 ± 536	1361 ± 487		1347 ± 381	1524 ± 452	1408 ± 463
$t_{1/2z}$ (h)	11 ± 5.6	8.0 ± 2.7	9.4 ± 2.9		9.6 ± 2.6	13 ± 13	9.4 ± 2.7
E1 parameters							
C_{max} (pg/ml)	373 ± 164	442 ± 196	414 ± 192		353 ± 157	405 ± 177	399 ± 189
AUC_{24}exo (pg/h·ml)	1135 ± 724	1482 ± 564	1449 ± 574		1114 ± 367	1552 ± 564	1616 ± 750
$\frac{AUC_{24}\text{exo E1}}{AUC_{24}\text{exo E2}}$	0.84 ± 0.32	0.99 ± 0.32	1.1 ± 0.28		0.84 ± 0.25	1.0 ± 0.27	1.2 ± 0.38

Values are the mean ± SD.

AUC_{24}exo, Area under the predicted concentration *vs.* time curve of exogenous compound calculated as AUC_{24}exo = dose/(predicted apparent total clearance). For d 1 of each cycle, AUC_{24}exo = AUC_0-∝exo; C_{min}, predicted minimum total serum concentration, just before an administration; after single administration, predicted concentration just before the second administration (C_{24h}); C_{max}, predicted maximum total serum concentration; $t_{1/2}$z, terminal half-life; WO, 2-month wash-out.

TABLE 2. E2 and E1 intra- and intersubject variabilities calculated over the six periods of serum collection

	Variability (%)			
	E2		E1	
	Intrasubject	Intersubject	Intrasubject	Intersubject
AUC_{24}exo[a]	23	27	41	36
C_{min}[b]	20	23	31	39
C_{max}	27	33	32	38

[a] For d 1 and 90 AUC_{24}exo is AUC_0-∝exo.
[b] For d 1 and 90, 24 h.

terest are reported in Table 1. Intra- and intersubject variabilities of the main PK parameters were higher for E1 than for E2 (Table 2).

Pharmacodynamics: FSH serum levels

Mean FSH levels measured before E2 administration were not statistically different ($P = 0.4$) in the postmenopausal women who had received E2 treatment compared with those who had received E2 and progestogen treatment (118 ± 7 and 117 ± 7 mIU/ml, respectively). Statistical analysis revealed no significant effect of either concomitant progestogen administration or treatment sequence on FSH values during the study. Consequently, these two factors were not taken into account in subsequent analyses, and data from the first and second treatment periods were pooled.

On d 1, the mean FSH serum level before E2 administration (predose) was 117 ± 5 mIU/ml and described a U time-concentration curve over a 24-h period (Fig. 2). The lowest FSH serum level was reached around 8 h after the administration of E2, with a value of 99 ± 4 mIU/ml, *i.e.* 16 ± 1% decrease. There was a statistical difference between the FSH levels measured at the different time points in the day ($P < 0.0001$): no change was observed between the mean values pretreatment and those up to 20 min after the intranasal administration of E2, but a continuous decrease was observed between 20 min and 8 h after E2 administration. This decrease was followed by an increase between 8–12 h (FSH levels representing 86% and 91% of the pretreatment value, respectively) and between 12 -24 h after the administration of E2. Compared with mean pretreatment value, FSH levels were different from 20 min to 12 h postadministration, but were not different at 24 h. The FSH profiles observed on d 14 and 28 were very similar to the profile observed on d 1. However, the mean pretreatment FSH serum level (for the combined data of all treatments) was significantly ($P < 0.0001$) reduced on d 14 and 28 compared with that on d 1 (−17% and −22%, respectively). Furthermore, the amplitudes of the decrease in FSH levels were reduced compared with that on d 1; maximum mean decreases were −18, −15, and −11 mIU/ml for d 1, 14, and 28, respectively. However, when these decreases were expressed as a percentage of the pretreatment FSH serum level, the amplitudes were quite similar throughout the 28-d cycle: −15, −16, and −12%, for d 1, 14, and 28, respectively.

A mechanistic-based PD model was used to describe the FSH data (Fig. 3). A summary of the baseline FSH concentrations (FSH_0), population and individual PD parameters and their variabilities is provided in Table 3. The good agreement between population and individual parameters supports the goodness of fits of the population PK/PD model (Fig. 4, A–D). The additive residual error term of PD model was estimated to be 4.8 mIU/ml (range, 4.2–5.3 mIU/ml).

The intrasubject variability in FSH levels calculated by ANOVA on untransformed FSH values at baseline and 8 h, when the maximal effect was observed, was low (<10%), and the intersubject variability in FSH levels was less than 42%.

Discussion

In the neuroendocrine regulation of the menstrual cycle, E2 exerts a negative regulatory feedback effect on pituitary FSH secretion and biosynthesis. This regulatory effect of E2 is exerted at two levels, directly at the pituitary level (17) and indirectly at the hypothalamic level via modulation of GnRH (18). It is well known that in normal women after the menopause, gonadotropin levels rise initially, then decline with age (19). Recently, Hall *et al.* (20) have shown that aging is also associated with a marked decrease in free α-subunit pulse frequency. This decrease provides evidence that the hypothalamic component of the reproductive axis can occur independently of changes in gonadal hormone secretion (20).

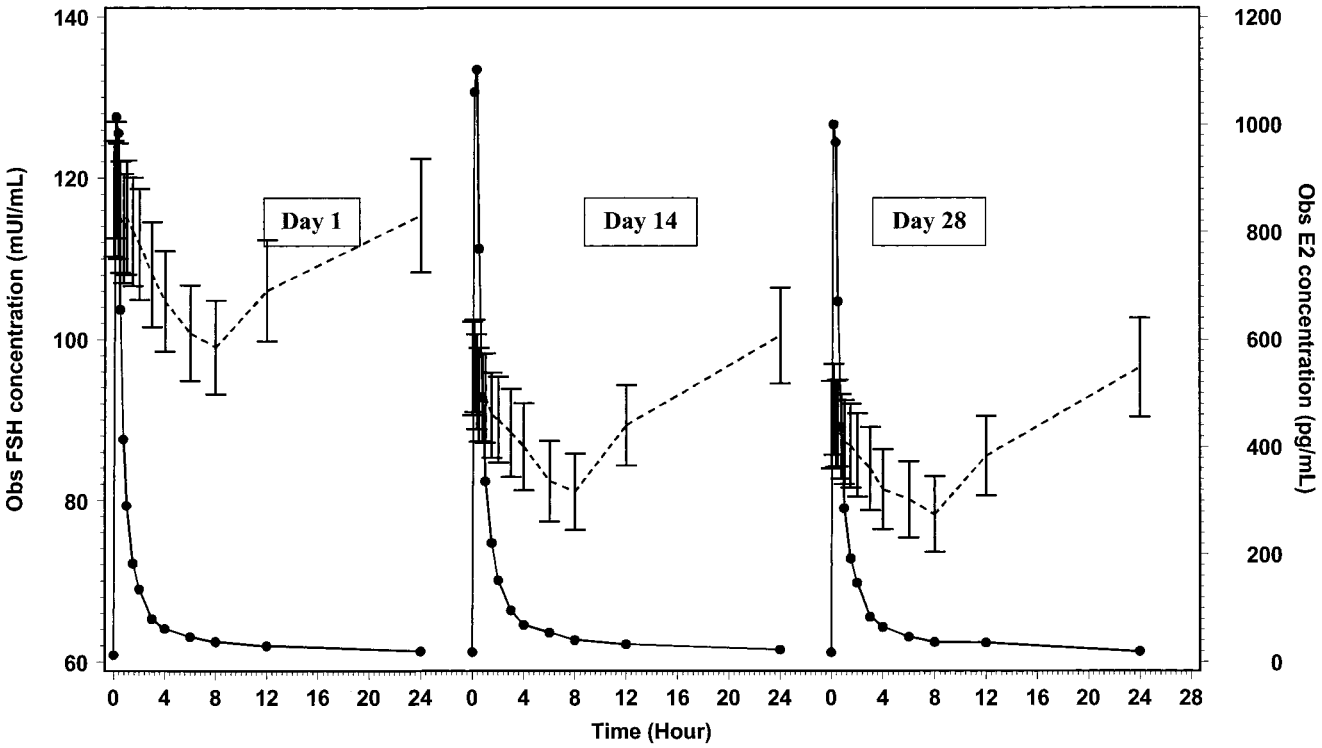

FIG. 2. Mean observed serum E2 (●) and FSH (error bars corresponding to SEM) concentrations *vs.* time on d 1, 14, and 28 of both treatments, with and without progestogen.

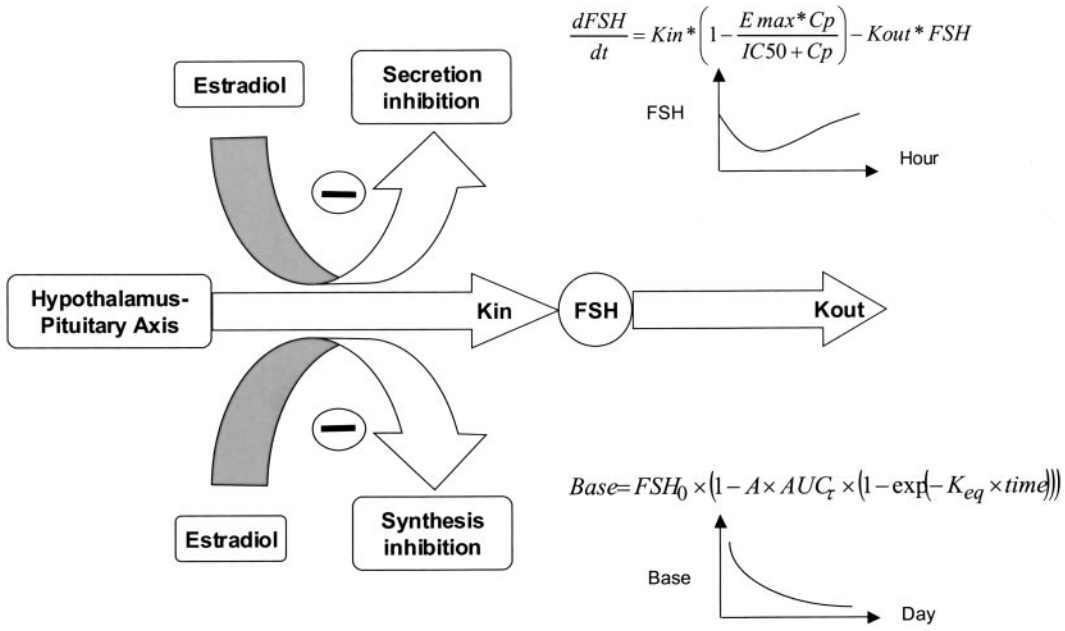

FIG. 3. FSH mechanism-based pharmacokinetic/pharmacodynamic model. K_{out}, First order rate constant for the loss of FSH; K_{in}, zero order constant for production of FSH; E_{max}, maximum effect attributed to E2; IC_{50}, concentration of E2 that produces half of the maximum inhibition; Cp, plasma E2 concentration; Keq, rate constant of equilibration; AUCτ, area under E2 time to concentration curve during a dosing interval.

Therefore, to avoid this age-related decline in FSH levels, the women included in the current study were at least 3 yr postmenopausal, but were younger than 65 yr of age. It is well established that oral and transdermal estrogen treatments decrease FSH levels in women (11). However, this is the first report of the PD response of FSH during estrogen replacement therapy using nasal administration of E2 in postmenopausal women. FSH pulse studies were not performed, as pulsatile FSH release is difficult to assess due to the slow metabolic clearance of the hormone (21, 22).

In relation to its PK, the effects of E2 on FSH after intranasal administration appear to be rapid and transient. A decrease

TABLE 3. FSH pharmacodynamic parameters and their variabilities

	Parameter		Variability[a]	
	Population estimate	Individual (median)	Intrasubject	Intersubject
FSH_0 initial (mIU/ml)	119	121	5.8	41
A constant (ml/pg·h)	0.00015	0.00018	60	84
K_{eq} (1/d)	0.099	0.088	86	105
K_{out} (1/h)	0.19	0.19	9.4	19
E_{max}	0.26	0.25	6.7	14
IC_{50} (pg/ml)	36	38	16	28
Hill factor	4.4	4.4		

[a] Variabilities calculated by ANOVA on individual model parameters over periods 1 and 2. FSH_0 initial, Baseline value of FSH before the first E2 administration; A constant, multiplied by AUC_τ gives the percent maximum decrease in FSH_0 from FSH_0 initial; K_{eq}, rate constant of equilibration; K_{out}, first order rate constant for the loss of FSH; E_{max}, maximum effect attributed to E2; IC_{50}, concentration of E2 that produces half of the maximum inhibition.

in plasma FSH levels occurs within 20 min, reaching the maximum decrease compared with baseline after 8 h, with a return to basal FSH levels at 24 h. In addition, intranasal E2 administration does not seem to lower FSH values to those of premenopausal women, which has been reported with other estrogen treatments (19). Whereas the results of the current study illustrate transient and short-term effects of pulsed E2 exposure, more sustained effects on FSH have been reported with other routes of administration where E2 exposure is maintained throughout the day, such as with the oral (23) and transdermal (9, 10, 11) routes. Studies performed with administration of 2 mg micronized orally administered E2 have shown a decrease in FSH serum levels, as observed after intranasal administration. However, in contrast to the intranasal route, FSH levels were still significantly suppressed 24 h after oral E2 administration (7). These differences in FSH serum profiles between the intranasal, transdermal, and oral routes are mostly explained by the differences in their plasma E2 profiles.

The present study provides further information than previously reported (12), especially about repeated administration, on the PK characteristics of E2 after intranasal administration. The results indicate that repeated treatments do not modify the PK of E2, thus there is a rapid increase in E2 serum concentrations within 15 min, which returns rapidly to about 10% of the peak value approximately 2 h after administration, and to untreated postmenopausal levels within 12 h. The PK of E1 and E2 were shown not to depend on the intranasal E2 timing of administration or on progestin coadministration. No accumulation of E2 (ratio for accumulation, ~1.1) and a slight accumulation of E1 (ratio for accumulation, ~1.4) were observed. Unlike oral administration of E2, which results in a significant rise in the levels of the major metabolite E1 due to the so-called first pass effect, (16, 24), an E1/E2 systemic exposure ratio close to physiological values (1:1) was shown in the present study. The PK profile after intranasal E2 dosing led to an intra- and intersubject variabilities that were relatively low for the area under the predicted concentration vs. time curve of exogenous compound [AUC_{24}exo; calculated as AUC_{24}, exo = dose/(predicted apparent total clearance); for day 1 of each cycle, AUC_{24}, exo = $AUC_{0-\infty}$exo] of E2 (~23% and 27%, respectively). Intra- and intersubject variabilities were higher for the AUC_{24}exo of E1, reflecting variability due to the metabolite formation (41% and 36%, respectively). For both estrogens, intra- and intersubject variabilities were of the same magnitude, showing that the input phase was probably the main source of variability rather than the metabolism. In contrast, greater intra- and intersubject variabilities in systemic exposures were shown when E2 was administered either orally or transdermally (25, 26); poor absorbers have been reported with patches (27). The exposure to exogenous E2 after intranasal administration is, therefore, transient and reproducible.

Analysis of the serum FSH levels demonstrated that pretreatment serum FSH levels on d 14 and 28 were lower than pretreatment FSH levels observed on d 1. This decrease in pretreatment FSH levels over the 28-d cycle reflects the equilibrium at the cellular level between the different complementary mechanisms regulating FSH synthesis, degradation, and distribution of FSH isoforms. It is now established that the proportion of the different isoforms of FSH in the blood and pituitary tissue is affected by endocrine status (28). For example, estrogen exposure in menopausal women shifts the distribution of isoforms from acidic to more basic forms, which have a shorter half-life in the circulation. Furthermore, the relative proportion of the more basic FSH isoforms within the pituitary tissue increases with the duration of the exposure to E2. Consequently, the decrease in pretreatment FSH levels observed after repeated E2 therapy might be explained in part by an increase in more basic FSH isoforms.

Although our study does not enable us to distinguish between a hypothalamic or a pituitary E2 effect, according to the PK/PD model, we have shown that E2 modifies not only FSH secretion, but also FSH synthesis. A direct pituitary effect of estrogen on gonadotropin secretion has previously been demonstrated by Clarke and Cummins (29) using a model in long-term ovariectomized ewes in which the pituitary had been disconnected surgically from the hypothalamus. In this model, long-term (6 months) estrogen treatment had a negative feedback effect on the pituitary gland. Furthermore, plasma FSH concentrations were reduced by approximately 50% after E2 treatment (30). Short-term regulation of gonadotropin subunit mRNA levels by estrogen has also been reported in both ovariectomized/hypothalamo-pituitary-intact and ovariectomized/hypothalamo-pituitary-disconnected ewes (31). In these animals, FSHβ mRNA levels were found to be reduced 8 h after they had received estradiol benzoate im. These experiments demonstrated that estrogen feedback can cause rapid alterations in pituitary gonadotropin subunit mRNA levels. Furthermore, in this

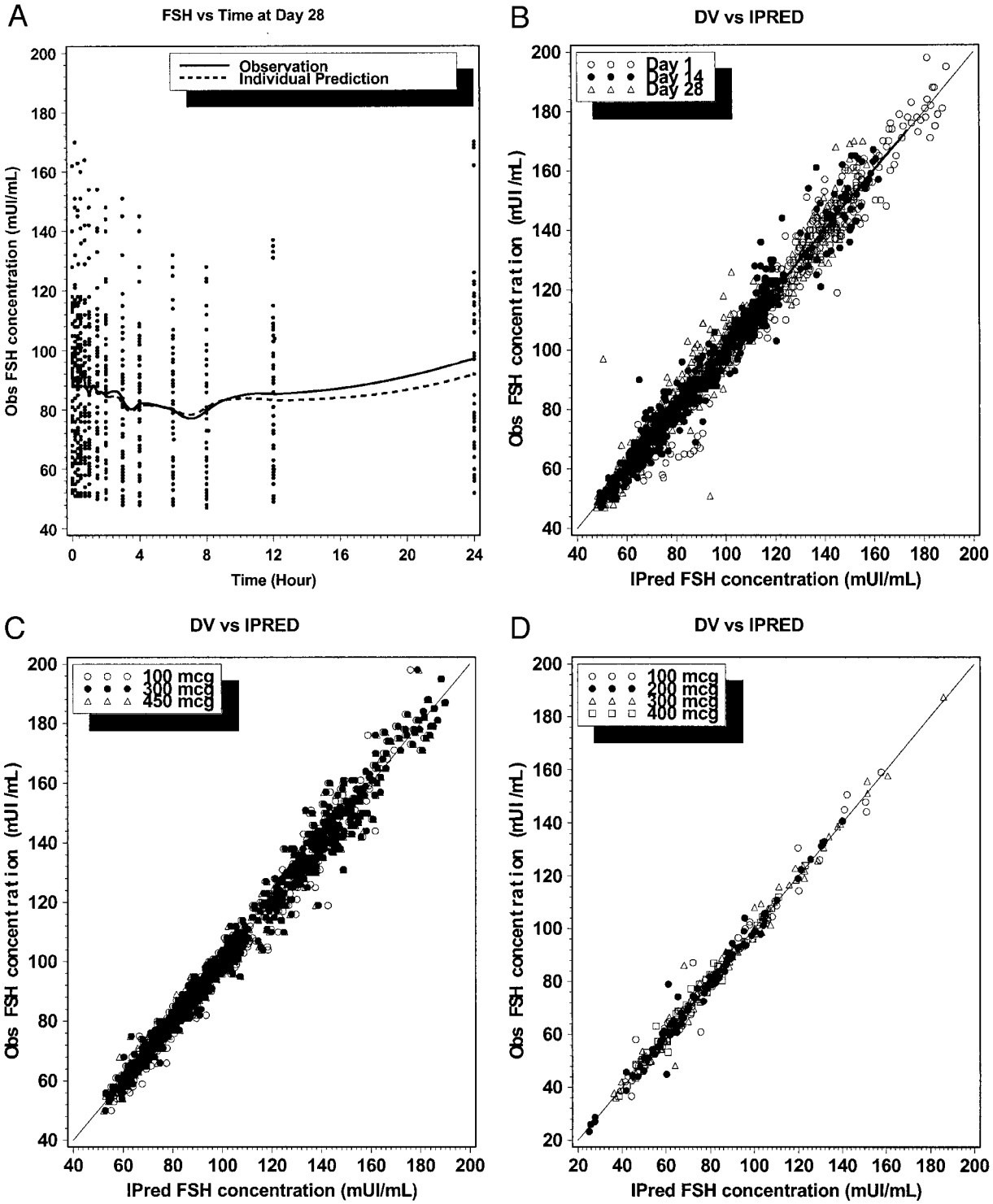

FIG. 4. FSH pharmacodynamic model: time course and goodness of fits. A, The *full* and *dotted lines* represent a cubic spline through the observed data and the individual model prediction, respectively. B–D, Observed (Obs) FSH concentrations *vs.* individual predictions (IPred) in different studies. *Lines* indicate identity. B, Results obtained in the present study after single and repeated intranasal administration at the dose of 300 μg. C, Model validation using data from a study (12) with single dose intranasal administration of 100, 300, and 450 μg. D, Model validation using data from a study (13) with intranasal administration at doses of 100, 200, 300, and 400 μg once a day.

model, short-term changes in FSHβ mRNA were also reflected by changes in FSH secretion. In the current study the effect of E2 on FSH secretion also appears to be reproduced from one cycle to the next. During the second cycle of treatment, FSH is even more sensitive to E2, although the plasma levels of E2 are comparable in all the cycles. As this effect is not related to either the route of administration or the bioavailability of intranasal E2 it is therefore likely to result from

an increase in E2 sensitivity of the hypothalamus or the pituitary.

A mechanism-based PD model linking the evolution of FSH levels to E2 concentrations was developed, taking into account the complex action of E2 on FSH. This model adequately characterized both the short-term (over 24 h) and mid-term (over 28 d) PD response after intranasal administration of E2 once daily for 28 d. Two PK/PD submodels were necessary to describe the dual actions of E2 on FSH. The first model (describing the inhibition of secretion that takes place within several hours) was an indirect inhibitory sigmoid Emax model relating the plasma concentrations of E2 to the serum FSH levels. The second model (describing the inhibition of the biosynthesis that takes place within several days) was a drug exposure response time model relating the E2 exposure to the predose values of FSH. The pulsed PK E2 profile observed after intranasal treatment is associated with a transient daily decrease in FSH, with a relatively low intrasubject variability compared with the intersubject variability. To challenge our integrated PK and physiological indirect PD model and validate the short- and mid-term effects of E2 on FSH concentrations, a Bayesian feedback estimation was performed on a single-dose S21400 study (12) and an S21400 dose-ranging study (13). The good agreement between the observed concentrations, the population, and the individual predictions through the different situations (doses, duration) supports the concept of short- and mid-term inhibitory actions of E2 on FSH levels.

Interestingly, dydrogesterone was not found to modify FSH levels. This finding is in accordance with previous results in premenopausal women (32) showing that dydrogesterone does not modify the basal level of FSH. Our data favor a low antigonadotropic activity compared with other progestrogens. Progesterone has been shown to induce a 56% decrease in FSH basal levels ($P < 0.0001$ compared with baseline) when administered with transdermal E2 treatment in postmenopausal women (11). The low antigonadotropic activity of dydrogesterone is confirmed by publications showing that it is unable to block ovulation (33–36).

In summary, PK analyses performed during this study in postmenopausal female volunteers showed that after intranasal administration the kinetic profile of serum E2 shows a transient exposure leading to pulsed estrogen therapy. This profile is associated with an E1/E2 ratio comparable to that seen physiologically and has low intra- and interindividual PK variabilities. The results of the current study also document the PD effects of repeated pulsed 17β-E2 treatment on the serum FSH profile; daily transient exposure to E2 elicits a transient decrease in FSH levels, which is thought to be related to direct FSH secretion regulation. Repeated treatment also produces mid-term effects, as shown by a progressive decrease in FSH basal levels during the treatment period, an effect that may be related to pituitary FSH synthesis. It thus appears that E2, when administered via pulsed estrogen therapy, may act on serum FSH levels through two distinct pathways.

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