Sex Specificity in Methadone Analgesia in the Rat: A Population Pharmacokinetic and Pharmacodynamic Approach

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Purpose. To quantify the extent to which a sex-specific dichotomy in the temporal evolution of the analgesic effect, after intravenous (i.v.) methadone injection in the rat, relates to the pharmacokinetics (PK) and pharmacodynamics (PD) that mediate the dose-to-effect pathway.

Methods. Tail-flick analgesia was measured after i.v. methadone injection (0.35 mg/kg) in female (n = 16) and male (n = 16) Sprague-Dawley rats. The PK were evaluated in separate female (n = 56) and male (n = 56) rats after they had received the same dose of methadone i.v. (0.35 mg/kg). A bicompartmental model described the kinetics and a sigmoid E_{max} model-related drug effect vs. simulated concentrations (pharmacodynamics) at the times of effect measurement. All model parameters as well as interanimal and assay variabilities were estimated with a mixed-effects population method using the program NONMEM.

Results. The area under the effect-time curve ($AUCE_{0\text{-}120}$) was (mean ± interanimal SD) 1859 ± 346 min in the females, which was significantly lower than the 4871 ± 393 min in the males ($P < 0.0001$). On the contrary, the profiles of concentration vs. time were higher in females and, therefore, corresponded inversely to the effect vs. time-relative magnitudes. The central volume of distribution, V1, was 1.94 ± 0.37 l/kg for female rats and 3.01 ± 0.33 l/kg for male rats. Also, the central clearance was 0.077 ± 0.006 l/min/kg and 0.102 ± 0.005 l/min/kg, respectively, for female and male rats. Both parameters differed significantly between sexes ($P < 0.0001$). The pharmacodynamic maximum observed effect parameter (E_{max}) was 37% ± 29% in female rats and 85% ± 16% in male rats, and these values were significantly different ($P < 0.0001$). The parameter for the concentration eliciting half of E_{max} (EC_{50}) was 24.1 ± 7.5 μg/l in female rats and 20.3 ± 2.9 μg/l in male rats, and the Hill-related exponent, γ, was 6.3 ± 3.9 in female rats and 5.5 ± 4.1 in male rats. These parameters did not differ significantly (at the $P < 0.05$ level).

Conclusions. A sex-specific dichotomy in the methadone antinociceptive effect, in the rat, was not proportionally related to plasma concentrations. Each sex corresponded to a distinct subpopulation of the PK parameters and one of the pharmacodynamic parameters (E_{max}). When the course of a drug involves PK or PD subpopulations, PK/PD modeling can afford the safest prediction of the effect-time evolution for a particular dose.

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INTRODUCTION

Animal studies indicate the existence of important sex-specific differences in opioid-induced analgesia as seen in rats and mice of the male sex, who display greater opioid analgesia compared with females, for several nociceptive models (1–4). This dichotomy in drug response could be secondary to changes in the dose-plasma concentration (Cp) relationship [pharmacokinetics (PK)], or the concentration effect [pharmacodynamics (PD)] relationship. Nevertheless, most of the relevant work is based on the dose-response curve, and therefore there is little information about the contribution of intermediary PK and PD.

Some research in male and female drug kinetics, both in animals and humans, has documented differences (5–7) that are typically attributed to changes in metabolism (8–10) and other factors (11). PD studies comparing males and females are scarce, in part because it is difficult to control the variability in effect measurements in animals or humans. In addition, females tend to show higher variability in the effect, possibly related to hormonal cycles. Therefore, males are typically preferred for drug-effect studies, particularly for analgesia studies, so even the fortuitous discovery of a sex-related principal component in the variability is unlikely.

Methadone is a μ-receptor opiate agonist widely used for perioperative and chronic pain control. Additionally, methadone is the treatment of choice for opiate withdrawal and is growing in use for first-line therapy for cancer pain (12–14). The PK/PD of methadone is characterized by high interindividual variability (15–17), the causes of which are still unclear. The drug is highly bound to α1-acid glycoprotein (AAG) (18,19) and is predominantly N-demethylated. The P450 cytochrome isoform 3A4 (CYP3A4) is the predominant enzyme involved in hepatic and intestinal metabolism of methadone in humans (20,21). Both metabolism and protein binding are sources of variability and could be sex-specific (5,22).

Although important differences between males and females have been reported for morphine and other opiates in the response to pain for a specific concentration, suggesting intrinsic PD changes (23,24), there is no information relating to methadone.

The pathway from dose to effect consists of PK and PD processes. In the present study we attempt to quantify the extent of sex-specific differences in this pathway for the rat after intravenous methadone. We obtain Cp and antinociceptive (analgesia) effect measurements after a single bolus methadone dose in groups of rats from both sexes. The observations are then analyzed with mixed effects modeling to obtain the true population distribution of the parameters, independently for each sex. Then statistical comparison is performed based on these distributions.

METHODS

Animals and Surgical Procedures

Methadone Administration by the Intravenous Route

The time course of the analgesic effect was evaluated in separate groups of female (n = 16; 211–243 g) and male

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Sprague-Dawley rats (n = 16; 183–260 g) after intravenous (i.v.) administration of 0.35 mg/kg methadone to all. Each animal had a complete effect evolution profile measured.

Also, a second set of female (n = 56; 225–250 g) and male (n = 56; 250–280 g) Sprague-Dawley rats received i.v. methadone (0.35 mg/kg) to assess the time course of methadone Cp. A destructive sampling, 1-point-per-animal design was used because of the quantification limit of the technique.

The day before the experiment, rats were lightly anesthetized with ether, and a polyethylene catheter (internal diameter, 0.3 mm; length, 10 cm; Vygon, Ecouen, France) was implanted in the right carotid artery for blood sample collection. Another catheter was inserted into the right jugular vein for the i.v. administration of methadone in both the male and female groups or in all animals. All catheters were filled with a solution containing NaCl 0.9% and 1% heparin (50 IU/ml; Chiesy Wassermann, Barcelona, Spain). The catheters were tunneled under the skin of the animal and externalized on the dorsal surface of the neck. After surgery, rats remained under fasting conditions but with *ad libitum* access to water (for 24 h).

The rats were supplied by the University of the Basque Country. The experimental protocol was approved by the Committee on Animal Experimentation of the University of the Basque Country. Animals were randomly distributed into groups of six and were kept under a controlled temperature of 20°C and a humidity of 70% with a normal 12-h light/dark cycle (8:00 am to 8:00 pm) for 1 week before any experiment was initiated. Food and water were available *ad libitum*.

Measurement of the Methadone Analgesic Effect Temporal Evolution

The analgesic effect of methadone was measured at 0, 2.5, 5, 10, 15, 30, 60, 90, and 120 min for male rats and at 0, 1, 2, 3, 4, 5, 10, 15, 30, 60, 90, and 120 min for female rats, after the i.v. administration of 0.35 mg methadone with the tail-flick (25) as the antinociceptive end point. Animals responded to a heat stimulus by moving their tail away from the focus of the stimulus, thereby exposing a photocell located under the tail. The reaction time was automatically recorded. The intensity of the heat was adjusted so that the basal measurements were of 2–5 s; animals with baseline latencies of longer than 5 s were excluded. A maximal cutoff time of 10 s was used to prevent tissue damage. the methadone analgesic effect was expressed as a percentage of the maximum possible (observed) response (MPR%) and was calculated as:

$$\text{MPR\%} = \left(\frac{\text{test latency} - \text{baseline latency}}{\text{cutoff time} - \text{baseline latency}} \right) \times 100$$

Drug Administration and Collection of Samples for the Methadone PK Assay

Rats of both groups were administered 0.35 mg/kg methadone, dissolved in saline solution, i.v. in 30 s. Blood samples (2.5 ml from each rat) were drawn at 1, 2, 3, 4, 5, 10, 15, 30, 60, 90, and 120 min after methadone administration, with each time point including four to six animals. Blood was placed in heparinized tubes, centrifuged at 2500 rpm for 15 min at 37°C, and the plasma kept frozen at –20°C until the assay of the methadone.

Methadone Assay

Plasma methadone levels were determined by high-performance liquid chromatography (HPLC) according to the method described by Wolff *et al.* (26), with minor modifications (27). Briefly, the HPLC equipment included an HPLC pump (type 422, Kontron Instruments, Bilbao, Spain) and an ultraviolet detector (Waters 486, Water Corp., Milford, Massachusetts). The separation was performed in a 5-μm silica column (Apex I, 25 × 0.45 cm, Teknocroma, Barcelona, Spain) and a Corasil Type II silica pre-column (Teknocroma). Methadone and the internal standard (benzhexol) were determined by a UV detector at a wavelength of 215 nm.

One milliliter of plasma was mixed with 10 μl of a solution containing the internal standard (1 μg/ml) and 0.5 ml of sodium carbonate buffer (1 M, pH 10) to which 5 ml of *n*-butyl chloride saturated in water were added for the extraction of methadone. The mixture was mechanically shaken for 15 min and then centrifuged at 4000 rpm at 4°C for 10 min. The *n*-butyl chloride upper layer was vacuum-aspirated, placed in a 5-ml glass tube, and evaporated at 50°C (AES 1000, Speed Vac Concentrator, Savant, Spain). The solid residue was dissolved in 110 μl of methanol, and 100 μl were injected into the chromatograph system. The mobile phase was methanol/1,2-dichloroethane/isopropanol/ammonium perchloride aqueous solution (100 g/l) in the proportion 90.5/5/4/0.5 (v/v) at a flow rate of 2 ml/min. The sensitivity of the analytic procedure is 5 ng/ml of methadone and is linear over the range of 5–350 ng/ml.

The unbound fraction in male and female rats (n = 6) was determined *in vitro* in 70 ng/ml of methadone in plasma at 37°C with the ultrafiltration technique using a micropartition system (Amicon MPS-1, Millipore Corp., Bedford, Massachusetts). There was no significant difference between the means of the two sexes (female rats, n = 6; male rats, n = 4; p = 0.3152).

Chemicals

Methadone (racemic mixture) chlorhydrate was supplied by Alcaliber (Madrid, Spain). Benzhexol chlorhydrate (dl-trihexyphenidyl) was obtained from Sigma (Madrid, Spain). All the remaining reactants and solvents were of analytic grade.

Pharmacostatistical Analysis

The nonlinear mixed effects method as implemented in NONMEM (nonlinear mixed effect modeling, NONMEM Project Group, University of California at San Francisco, California) was used for fitting compartmental PK and appropriate PD as well as statistical models to the observations to obtain estimates of the typical parameters and of the interanimal and assay variances. Alternative models were compared based on the NONMEM objective function (minus twice the log-likelihood [–2LLD]), the Akaike criterion for p parameters (AIC = –2LLD + 2p) and the visual inspection of plots of the residuals. The –2LLD is distributed as a χ^2-like variable, and a difference of 7.7 in –2LLD between models differing in a single parameter is significant at the $P < 0.005$ level. This was the level used for comparisons here.

Male and female rats were analyzed preliminarily as a single population with a bicompartmental model. The SEs of the parameter estimates were not obtainable under any sta-

tistical model for this population. Nevertheless, we proceeded with a tentative covariate analysis. Individual posterior Bayesian parameter estimates were obtained for that mixed population, but using a noninformative prior to exploring the existence of subpopulations. The covariate relation search of the parameters vs. sex and weight with a general additive model showed sex to be an independently significant categoric covariate for both apparent central (V1) and tissue volumes of distribution (V2). One hundred bootstrap replications of the observations (n = 112) showed sex to be included with a sex/weight frequency ratio of 60:16 for V1 and 14:7 for V2. The central clearance (CL) had a sex/weight frequency ratio of 31:7, and the intercompartmental clearance (Q) had a 36:49 ratio. The V1 and V2 covariate models were linear functions of sex only, whereas CL depended solely on weight. Q had a mixed model with sex and weight. Based on these results, and given the good size of the sample, we separated the rat population into two subpopulations (n = 56 for both) based on sex. Also, the PK model parameter estimation was standardized by the animals' weight.

PK

Mono, bicompartmental, and tricompartmental PK models were tested for fit to the data in both groups. There was a drop of between 36 (male) and 110 (female) points in the AIC when going from the monocompartmental to the next more complex representation (two compartments). A tricompartmental model showed an 11-point AIC drop in the female rats, but an 18-point increase in the male rats. The model for the females had difficulty converging to a minimum and was unable to identify the variability of the deep compartment parameters, so an SE estimate was not produced and the tricompartmental fit to the females was considered to be invalid. The bicompartmental is the most parsimonious model for both sexes.

Because this was a single-time-point-per-animal design, the *i*th rat's concentration at the only time point (subindex j = 1) was represented as:

$$Cp_{il} = g(p_i, t_{il}) + \varepsilon_{il} \quad (1)$$

where Cp_{i1} are the elements of the vector of concentrations in plasma and $g(p_i,t_{i1})$ is the bicompartmental structural model for parameter vectors $p_i = (V1, CL, V2, Q)_i$, one for each rat, with variances ω_p (p × 1 vector). Both p and ω_p are fixed effects, and t_{i1} is the vector of time elements. The residual "noise" matrix elements, ε_{i1}, were modeled as normally, independently, and identically distributed with mean $E[\varepsilon_{i1}] = 0$ and variance $Var[\varepsilon_{i1}] = \sigma_\varepsilon{}^2\, g(p_i, t_{i1})^2$, and a proportional model for heteroscedastic errors in which σ_ε is a fixed-effect coefficient of variation (CV) to be determined. This structure was selected after inspection of residuals and results from a structure in which the exponent of σ_ε was a parameter, and this was closer to 2 than to 0.

Preliminary tests of the parameter distribution with Bayesian estimates based on literature values showed normal-like distributions for V1, V2, and Q, whereas CL was left skewed (log-normal-like). Standardization of the parameters by each rat's weight changed the appearance but not the skewness of the distributions. Additive statistical models for the parameter variability consistently failed due to negative values in the process of estimation, so a log-normal model for the parameters was used for the random-effect elements as follows:

$$p_i = \bar{p} \times e^{\eta\_pi}$$

where $\bar{p}$ is the typical population value for each parameter for males and females, and η_p is the random effect for the parameter. This models results in variance estimates, ω_{ii}, which are CVs for the parameters.

The NONMEM first-order conditional estimation (FOCE) method with a full covariance structure was first attempted but the algorithm failed to converge successfully (i.e. producing standard errors for the estimates) independent of the error or covariance structure used. In contrast, the first-order approximation provided successful estimates with a five-significant-digit requirement in the parameter estimation. There were insignificant differences in the parameter estimates between the two methods for the same pharmacostatistical model and variance structure, although the FOCE did show higher sensitivity to outliers in the data, which were seen as failures to converge or large changes in the partitioning of variabilities. There was no significant change in the objective function between successive runs with altered covariance structures, and a diagonal structure finally was chosen.

In population modeling of destructive sampling data, the partition of the variability in its interanimal (ω_p) and intra-animal (σ_ε) components introduces a positive bias in the parameter variances (28). Because we are testing the null hypothesis of no significant difference between the population mean estimates for male and female animals, the positive bias in the SD was not seen as problematic because it protects against a false rejection (or a type II error).

PD

Methadone analgesia is related to concentration by an E_{max} sigmoid model:

$$E_{ij} = E_{max\,i} \frac{Ce_{ij}{}^{\gamma\_i}}{EC_{50i}{}^{\gamma\_i} + Ce_{ij}{}^{\gamma\_i}} + \varepsilon_{ij} \quad (2)$$

where E_{ij} is the analgesic effect observed in the *i*th rat at the *j*th time point, corresponding to simulated concentrations Ce_{ij} at the effect site; γ_ι is the Hill sigmoidicity measure of the effect-vs.-concentration curve for the *i*th animal; E_{max_i} is a parameter designating the maximum possible effect; and the parameter EC_{50-i} is the concentration eliciting 50% of E_{max}. The residual "noise" matrix elements, ε_{ij}, were modeled as multivariate-normal and independently and identically distributed with mean $E[\varepsilon_{ij}] = 0$ and variance $Var[\varepsilon_{ij}] = \sigma_\varepsilon{}^2$ for homoscedastic or additive errors evaluated similarly as for the PK.

If the plasma or central compartment is at instantaneous equilibrium with the effect site, then Ce = Cp, always. If there is disequilibrium, then the evolution of the effect can be seen physiologically and/or graphically to lag behind that of Cp. In that case a "link" model can be used to arrive at estimates of Ce via the addition of an effect-site compartment. Although this lag appears frequently in the concentration contrasted with the effect evolution (and so it does for methadone), it is not always identifiable due to masking by the variability.

To explore the lack of equilibrium, a third kinetic compartment is typically used to model the effect-site kinetics. An infinitesimally small volume is assumed for the site, so losses to it are not compensated in the central compartment equation. We propose a general expression consisting of a simple creation-destruction equation describing biophase PK, as follows:

$$\frac{dCe}{dt} = \lambda - \mu \equiv \sum_{i=1}^{n} q_i Cp - \sum_{j=1}^{m} k_j Ce \qquad (3)$$

where λ and μ, respectively, are creation and destruction rates of Ce. Equation 3 is a heuristic closure expression in a system of equations that is used to "link" the classic bicompartmental model for Cp with Eq. 2 for the effect. Thus, it accounts for the delay between the appearance of the central concentration and the effect peaks. A likely representation then is that on the right-hand side, where Cp is plasma concentration and q_i represents the creation probabilities or transfer rates from plasma to the effect site corresponding to *n* physiologic reasons for a disequilibrium and *m* processes for dissociation from the effect site without a return to plasma. The two rate totals do not necessarily correspond to the same physiologic process. In practice, however, it is difficult to identify deep compartment rates, so, without loss of generality, we group all physiologic rates into one ($n = m = 1$) and assume for simplicity that they are equal, that is, $q_1 = k_1 = k_{e0}$, which is an equilibration constant that typically is used in the literature to characterize the half-life of equilibration between plasma and the biophase (29). Both PK/PD with Eq. 2 and Ce = Cp and a link model with Eq. 3 were tested here.

Coupled PK/PD simulations were performed by Monte Carlo (random) sampling from the bicompartmental model parameter distributions and using the typical parameters of Eq. 2 with Ce = Cp (no disequilibrium). The kinetics were individualized by weight for the same triple consecutive-dose regimen in three male and female rats.

Statistics

Statistical comparison was performed with the parametric unpaired *t* test with Welch's correction for unequal variances at the $P < 0.05$ level, at least. The tests were performed between typical population parameters for the two sexes, using their interanimal SDs as the spread measure, and also between the means of concentration and effect observations at specific time points between animals, with their corresponding SEs used as the spread measure.

RESULTS

Methadone-Induced Antinociception

A single i.v. dose of 0.35 mg/kg methadone in the rats produced a marked sex-related dichotomy in antinociceptive activity observed through the tail-flick test. In Fig. 1, the analgesic effect in males (MPR% mean with SE bars) reaches a maximum of 88% at 10 min after injection. In contrast, the MPR% in females was nearly half that in males (42% at 10 min) at the time of peak antinociception, after the same dose. Analgesia was significantly higher in males at 5, 10, 15, 30, 60, and 120 min after injection (Fig. 1).

The (mean ± SD) overall magnitude of antinociception, as reflected in the area under the time-action curve ($AUCE_{0\text{-}120}$) was 1859 ± 346 min in the females, which was significantly lower than the 4871 ± 393 min in the males ($P < 0.0001$). Baseline analgesic values did not differ between the two groups.

PK

Figure 2 shows the mean and SEs for the time evolution of Cp values for a single-point-per animal measurement design after i.v. injection of 0.35 mg/kg methadone. Measurements in the initial phase were significantly higher in females at 1 min ($P = 0.03$), 2 min ($P = 0.001$), 3 min ($P = 0.035$), 4 min ($P = 0.004$), and 5 min ($P = 0.008$), with no significance between the other time points. Table 1 shows the bicompartmental population model parameter estimates for both the females and males standardized for their weight. All parameters are significantly different between the two sexes. The females show larger interanimal variability (CV% = 100 × SD/mean, CV%) in the central compartment parameters, es-

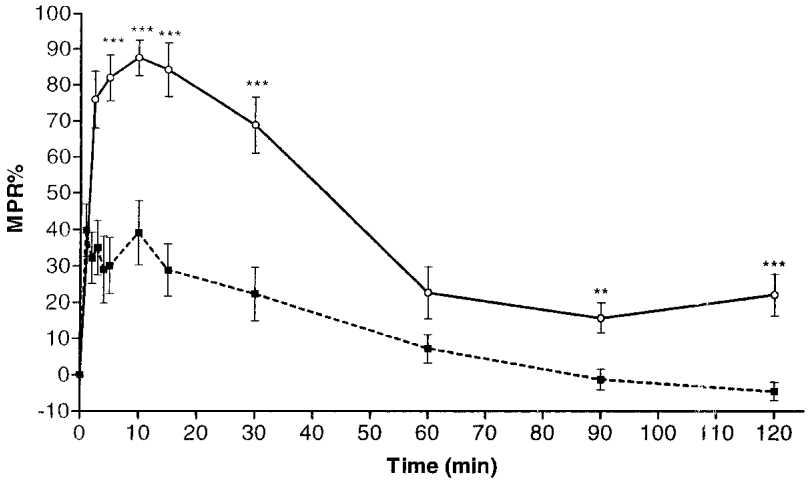

Fig. 1. Temporal evolution of analgesic effect (tail-flick), after i.v. injection of 0.35 mg/kg methadone in male Sprague-Dawley rats (n = 16; open circles with solid line) and female Sprague-Dawley rats (n = 16; solid squares with dotted line). Observation means and SEs are shown for the effect obtained as MPR%. ***, $P < 0.0001$; **, $P = 0.0014$).

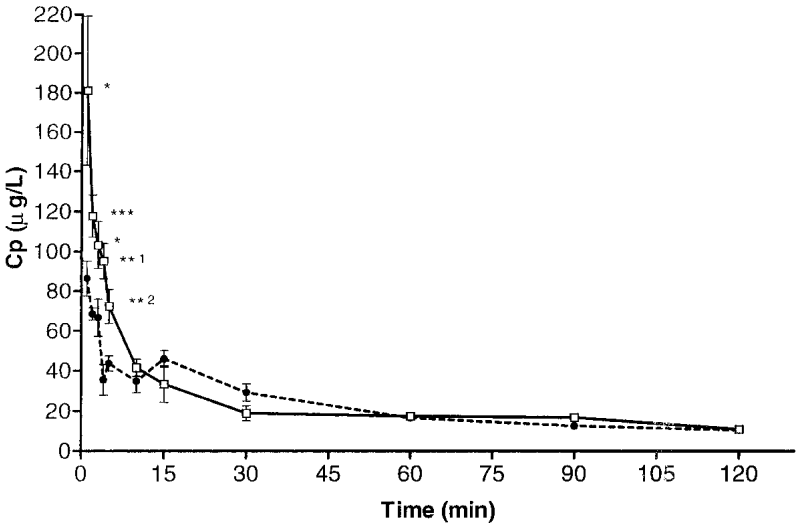

Fig. 2. Cp vs. time, after i.v. injection of 0.35 mg/kg methadone in female rats (n = 56; open squares with solid line) and male rats (n = 56; solid circle with dotted line). Measurement means and SE bars are shown. *, $P = 0.03$; **[1], $P = 0.004$; **[2], $P = 0.008$; ***, $P = 0.001$.

pecially in V1, the possible reasons for which are discussed later. The males had large interanimal variabilities in the deep compartment parameters (i.e., V2 and Q).

PD

The effect observations represented graphically show an apparent delay in the effect with respect to the Cp time course. Based on both the assumption of Cp = Ce (i.e., no additional effect compartment or Eq. 2 alone) and that of nonequilibrium (i.e. Eq. 2 and Eq. 3 linked to the PK) were tested for a fit to the effect time course observations. The AIC difference between the more complex and the simpler model was 12 for the females and 3 for the males, marginally in favor of the k_{e0} link model. However, the parameters were not identifiable in the more complex model within the NONMEM iteration and had to be estimated by attempting values for the exponent of Eq. 2 as a fixed parameter that minimized a series of consecutive runs. The equilibration constants estimated with the effect compartment model above were (population mean ± interanimal SD (standard error of the estimate [SEE]) $k_{e0} = 0.18 \pm 0.05$ min (0.047 min) for the male and 1.20 ± 4.60 min (1.18 min) (i.e., undefinable) for the female with the Hill exponent fixed through the fitting. The simpler model with no disequilibration and Ce = Cp was selected because it provided the overall best explanation of the observations.

Concentrations were simulated based on the results listed in Table 1 at the times of effect-time observations using the population predictions without a random component. Individualization was achieved with the weight of the animals as a covariate. Table 2 lists the PD parameter estimates. The males show significantly higher E_{max}, but insignificantly different potency (EC_{50}) and Hill exponent. All parameters in the females show large interanimal variability. The PD relationship is depicted in Fig. 3 as the model-predicted MPR% analgesic effect vs. the simulated concentrations for the parameters in Table 2. The temporal evolution of the effect, after a single administration of 0.35 mg/kg, as predicted by the integrated population PK/PD model with the weight as a co-

Table I. Typical Population PK Parameter Mean Estimates with SEEs from the NONMEM First Order Method[a]

	Female[b]			Male[c]			
Parameter	Mean (SEE)	SD	CV%	Mean (SEE)	SD	CV%	*P* value[d]
V1 (l/kg)	1.94 (0.20)	0.37	19	3.01 (0.43)	0.33	11	<0.0001
CL (l/min/kg)	0.077 (0.009)	0.006	7	0.102 (0.006)	0.005	5	<0.0001
V2 (l/kg)	8.22 (1.53)	3.24	39	4.53 (1)	3.03	67	<0.0001
Q (l/min/kg)	0.30 (0.03)	0.02	22	0.58 (0.15)	0.44	76	<0.0001
σ_ε (CV%)	19% (29%)			15% (12%)			

[a] Interanimal variability is expressed both as SD and as CV% for female and male Sprague-Dawley rats after i.v. injection of 0.35 mg/kg methadone. The significance test is between means. The interassay variability is reported as the CV for a proportional error model (see text). n, the number of animals; m, the total number of observations.

[b] -2LLD = 318.442; n = m = 56.

[c] -2LLD = 336.166; n = m = 56.

[d] By *t* test.

Table II. Typical Population PD Parameter Mean Estimates with SEEs from the NONMEM First Order Method[a]

	Female[b]			Male[c]			
Parameter	Mean (SEE)	SD	CV%	Mean (SEE)	SD	CV%	*P* value[d]
Emax	37 (7.5)	29	80	85 (5)	16	19	<0.0001
EC50 (μg/L)	24.1 (2.3)	7.5	40	20.3 (1.5)	2.9	14	0.07[e]
γ	6.3 (0.9)	3.9	62	5.5 (1.9)	4.1	75	0.57[e]
σ_ε	15 (6.7)			22 (10)			

[a] Interanimal variability is expressed both as SD and CV% for female and male Sprague-Dawley rats after i.v. injection of 0.35 mg/kg methadone. The significance test is between means.
[b] -2LLD = 1212.713; n = 16; m = 176.
[c] -2LLD = 959.195; n = 16; m = 128.
[d] By *t* test.
[e] Not significant at $P < 0.05$.

variate, is shown in Fig. 4. The mean observation lines for the two populations of rats are overlaid with the simulation points.

Figure 5 shows simulations of the arrival at a steady state for three individual rats of different weights for each sex (male, Fig. 5a; female, Fig. 5b). The protocols represented are three doses of 0.35 mg/kg methadone administered i.v. over 2 min at 40-min intervals. The depiction is a single realization from the distribution of PK/PD parameters estimated in the previous steps, hence the interindividual variability seen in the Fig. 5. At steady state, similar Cp values are reached for both groups, but the effect remains reduced in the female.

DISCUSSION

A sex-specific dichotomy was observed primarily in the overall pharmacologic effect of methadone, when a previously validated (2,24,27,30) tail-flick analgesia model was used on young Sprague-Dawley rats of both sexes ($AUCE_{0\text{-}120}$: female rats, 1859 min; male rats, 4871 min). This result is consistent with reported sex differences in antinociception induced by morphine and other opiate drugs (1,2) including opioid peptides (31).

Other studies based on dose-response curves, rather than on integrated PK/PD analyses, have observed that males have greater effect response per dose (i.e., smaller ED_{50}), inferring an intrinsic sex-related difference in antinociception induced by endogenous and exogenous opioids. However, this cannot be generalized to other opioids, because no differences were seen, for example, in fentanyl and buprenorphin (23). The mechanism underlying sex differences in opioid analgesia remains elusive. One possibility is that opioid PK may differ between the sexes (24,32). In fact, there is evidence that sex may play a role in the PK of many other drugs (5–7).

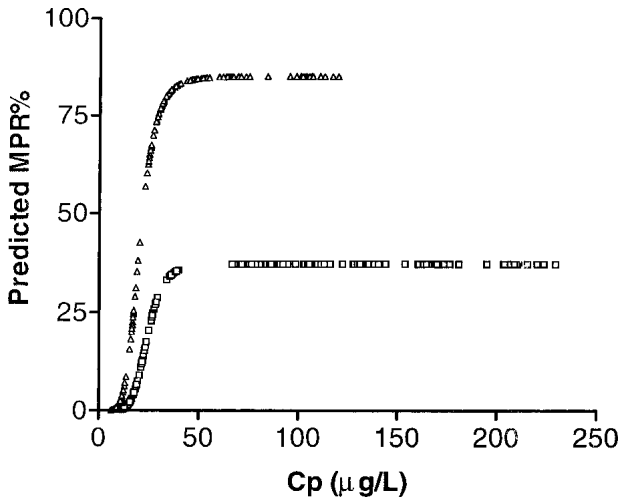

Fig. 3. Typical (mean) population fit for the analgesic effect as MPR% vs. Cp.

In this study, we found that after 0.35 mg/kg methadone was injected i.v.into the rat, the analgesic effect, which was higher in males, corresponded inversely to lower Cp values in males and higher values in females. Subsequent population-integrated PK/PD screening, on separate male and female groups, showed that the principal parameters differed significantly.

Our PK results, showed clear differences in the plasma disposition of methadone. These changes were found in both the distribution and elimination processes and led to a different evolution of the concentration, which was higher in the females (C_{max} = 181.10 μg/l in females vs. 86.35 μg/l in males). There are no data in the literature regarding methadone kinetic differences between sexes in the animals, but in a recent population-based study of oral methadone in opiate addicts, differences between human males and females were observed in bioavailability (f) corrected volume of distribution (vd) when sex was included as a covariate (33). In another similar study, Vol at steady state (V_{ss}) was found to be higher in female opioid human users than in male users (20). Although those authors found no difference in clearance, our results here showed a slight yet significant change in this parameter. This could be attributed to the difference in the kinetics of methadone between rats and human postaddict volunteers, with half-lives of 70–90 min (34) vs. 19–58 h (35), respectively, and different metabolic routes.

The parameter estimates in male rats coincide with those previously reported for that animal model (27,34). The interanimal variability in the females was approximately four times that of the males in the central compartment parameters and is possibly due to hormonal cycles affecting the metabolism of the former (36,37).

The dichotomy observed in the PK could be due to multiple causes. First is body composition. It is known that male

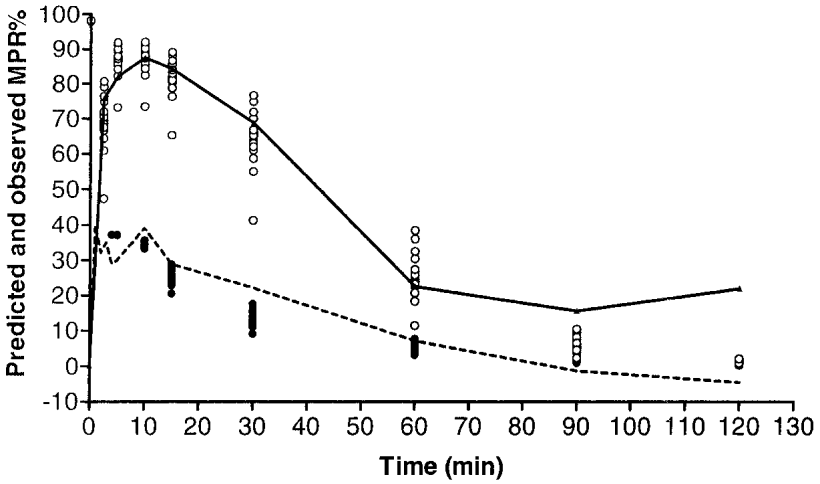

Fig. 4. Observation means of evolution (males, solid line; females, dashed line) of the analgesic effect with individual population predictions (males, open circles; females: solid circles) overlayed after a single dose of methadone (0.35 mg/kg).

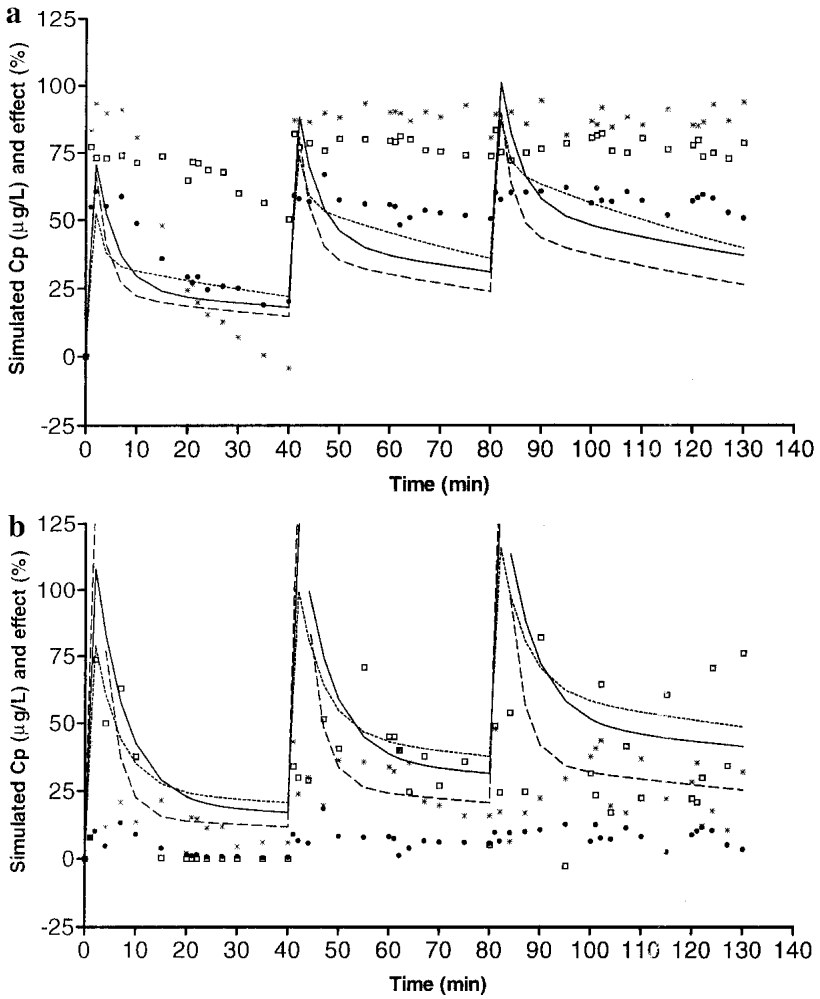

Fig. 5. Coupled PK/PD simulation of concentration (Cp, lines) and effect (MPR%, symbols) evolution for a single Monte Carlo realization of the population PK parameters and a 20% noise error. Instantaneous equilibrium between plasma and the biophase is assumed. The progress of an identical regimen of three repeated weight-adjusted bolus doses of methadone (0.35 mg/kg) is shown in three males (a) and three females (b) with a clearly observable sex-related deviation in PK and the MPR effect. Individual pairs are: solid line with solid circles; dashed line with open squares; and dotted line with star symbols.

mammals have different body structure compared with female mammals, as is reflected for example in mean weight and possibly also in body water spaces, muscle mass, blood flow, and organ function, and can therefore also affect the PK parameters of many drugs (38). Women have relatively higher body fat (connected with our V2) than do men, which can alter the volume of distribution at least of lipophilic drugs (39,40). Second is protein binding. Methadone is highly bound to α1 acid glycoprotein, and the levels of this protein seem to be slightly lower in females than in males (11). Protein binding was assessed, and no significant differences were found between sexes, so changes in distribution are not due to changes in the unbound fraction. Third is metabolism. It is well-known that cytochrome P450 activity is greater in males than in females (9). Male rats have relatively high expression of an isozyme of cytochrome P450 in the 3A family (CYP3A4), whereas female rats have extremely low levels of this isozyme (9). A similar sex difference is not, however, observed in humans (41), hence an extrapolation to humans is not straightforward. But the results of animal studies can be used to illustrate the fact that significant sex differences can occur in mammals, at least in drug metabolism and elimination, and can provide the impetus for sex-based research in humans.

The sex difference observed in our PK screen, although possibly due to one of the above processes, still would not explain the observed inverse relationship between the temporal evolution of concentrations and effect in each sex. And this is in agreement with the literature on other opiates.

In one of the few studies (42) including data related to the kinetics (i.e., access to the brain), it was observed that the difference in the drug effect for morphine in male mice seems to be due to a greater availability of morphine to the brain, suggesting sex differences in drug disposition. Other authors have rejected the influence of PK because after intracerebroventricular (31) morphine administration and after microinjection of morphine to the rostral ventro medial medula, both in rats, there were still sex-specific differences in opioid analgesia (42,43). Additionally, in another study (2) no sex differences were observed in the peak levels of morphine in the brain and in serum at the time corresponding to the peak analgesic effect. In a following study of the same authors (3), no dichotomy was seen in the elimination half-life of morphine in plasma, and neither were there any differences in the disappearance of morphine from the brain. On the basis of these results, the authors suggested that the sex-specific differences observed between males and females in the response to the antinociceptive activity of morphine cannot be explained by the PK of morphine. They suggest that this could be inherent to the differences in the sensitivity of the brain to morphine. But none of these studies employed an effect-vs.-concentration approach (i.e., PD).

It should also be noted that most of the earlier reports discussed here concern morphine, which has a different PK than methadone. For example, a very important difference is that morphine has active metabolites that alter the interpretation of the results. Instead, it seems that methadone gives rise only to inactive metabolites (44). Methadone, however, is a racemic mixture of two enantiomers with enantioselective PK and only one active isomer (45,46). In this study, as well as in most clinical and animal studies, methadone is administered as a racemic mixture, so the ratio of enantiomers in plasma could differ between sexes, and that could explain the alteration in the PD (47).

Beyond the PK, other explanations are feasible for the lack of relationship between the temporal evolutions of concentration and effect. First, *P-glycoprotein* (P-gp) is an adenosine triphosphate-dependent drug efflux pump that is expressed in several human tissues including the blood-brain barrier (BBB). P-gp expression in the BBB seems to be influenced by sex, inasmuch as P-gp levels are 40% higher in females than in males (48). Interactions of methadone with P-gp have been suggested (49). It also has been observed that the analgesia of morphine and methadone is greater *in vivo* in knockout mice (i.e., mice lacking P-gp expression) (50). This could explain the fact that our female rats show less effect with higher concentrations compared to male rats. The second explanation is a lack of equilibrium between Cp and drug effect (k_{e0}). This parameter depends on multiple factors such as blood flow, the BBB, diffusion of the drug to brain sites containing μ-opioid receptors, receptor-binding interactions, and possibly neuronal dynamics. Our tentative observations in the rat coincide with previous results (male rats) (27) showing faster equilibration for the female rats (higher k_{e0}). There is no literature comparing males and females for analgesia with opioids, although for other drugs (51) and effects (e.g., for morphine and respiratory depression as the end point), sex-related changes in the equilibration rates moved in the same direction (52).

With respect to our PD results, we found no sex-specificity in EC_{50}. But, despite the large interindividual variability in the females, the E_{max} was significantly smaller for that sex ($P < 0.0001$), so there is less effect for the same concentration.

These intrinsic PD changes, which are independent of the kinetics, can be explained by various mechanisms, among which are differences in the number and regional distribution of opioid receptors in "sexually dimorphic" brain regions in males and females (53), or sex differences of the endogenous neurochemical systems that participate in the analgesic response (54). Nevertheless, there is controversy, and some authors have indicated that there are no differences between male and female rats in brain μ- or δ-opioid receptor densities (55).

Given that methadone is administered in repeated doses, we have performed an integrated PK/PD simulation in males and females starting with the same dose and regimen for both sexes. There was a marked difference in the effect at steady state, contrasting with the similarity in the Cp.

Our results show the importance of the integrated PK/PD study, which covers the complete pathway from dose to effect, in providing a more complete picture of the sources and significance of all variability. Particularly regarding the clinical use of methadone, where the dose or Cp is a surrogate for the effect, a sex-specific lack of a simple linear connection in the pathway could easily lead to inappropriate dosing.

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