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Altered Plasma and Brain Disposition and Pharmacodynamics of Methadone in Abstinent Rats

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ABSTRACT

The pharmacokinetics and pharmacodynamics of methadone were investigated in control and abstinent rats. Minipumps filled with saline (control group) or saline-morphine (abstinent group) solutions were used to induce physical dependence. Solutions were delivered continuously by minipumps for 6 days. The physical dependence was evaluated 12 h after minipump removal by measuring specific withdrawal signs. Animals from the abstinent group showed clear withdrawal signs such as hostility on handling and weight loss. Plasma and brain disposition and pharmacodynamics of methadone were evaluated after a 0.35 mg/kg i.v. bolus dose administered 12 h after minipump removal. Plasma clearance, distribution clearance, and volume of distribution at steady-state were significantly decreased ($P < 0.05$) in the abstinent group. Plasma levels of α_1-acid glycoprotein and plasma protein binding were significantly increased ($P < 0.05$) in the abstinent group. The estimates of pharmacokinetic parameters based on unbound plasma concentrations did not differ between groups, with the sole exception of the unbound apparent volume of distribution. The access of methadone to the brain was significantly faster ($P < 0.05$) in the abstinent group, although the extent of distribution in the brain was diminished in comparison with the control group. Analgesia recorded with tail-flick was used as the pharmacodynamic endpoint. Analgesic response and effect compartment concentrations of methadone were related by the sigmoidal E_{max} model. Estimates of C_{50} [steady-state plasma concentrations eliciting half of maximum effect (E_{max})]] based on unbound concentrations did not differ between groups. On the other hand, the estimate of E_{max} had decreased by 65% in the abstinent group.

The study of the pharmacokinetic-pharmacodynamic (*pk*/*pd*) relationships of opioid drugs can be hampered by various factors such as 1) the development of acute and/or chronic tolerance (Ekblom et al., 1993; Gårdmark et al., 1993), 2) disease (Inturrisi et al., 1987), 3) withdrawal syndrome (developed after stopping continuous exposure to an opioid; Adams and Holtzman, 1990), and 4) cross-tolerance phenomena (Paronis and Holtzman, 1992). Among all these, only the development of tolerance has been studied to some extent under the *pk*/*pd* perspective; recently, models have been proposed to account for the development of tolerance for morphine (Ekblom et al., 1993; Gårdmark et al., 1993; Oullet and Pollack, 1995) and alfentanil (Mandema and Wada, 1995) in healthy (control) rats following different modes of drug administration. Less attention has been paid to the effect of disease on the *pk*/*pd* properties of this kind of drug, although Inturrisi et al. (1987) explore by means of a *pk*/*pd* model the analgesic effects of methadone in cancer patients.

Regarding withdrawal syndrome, it is well known that this status can produce physiological alterations such as weight loss (Goode, 1971), changes in plasma protein level (Garrido et al., 1996), diarrhea (Goode, 1971) etc., all of which may modify drug kinetics. To our knowledge, the disposition of opioids has not been characterized with regard to such a syndrome; there are studies in which the plasma and brain levels of the opioid methadone were compared between abstinent and healthy rats (Liu et al., 1983), but no pharmacokinetic models were proposed.

In addition, drug efficacy and/or drug potency are likely to be altered during withdrawal because there has been a previous exposure to an opioid (Paronis and Holtzman, 1992); for example, the drug potency, evaluated as ED_{50}, was increased for fentanyl and etorphine, after a previous 3-day exposure to morphine pellets (Brase, 1986). Although the response to opioids in opioid-treated rats has

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ABBREVIATIONS: *C*, total plasma concentration of methadone; C_u, unbound plasma concentration of methadone; C_{brain}, total brain concentration of metadone; $C_{50,}$ steady-state plasma concentrations eliciting half of maximum effect (E_{max}); *pk*/*pd,* pharmacokinetic-pharmacodynamic; f_u, unbound fraction; *AAG*, α_1-acid glycoprotein; %MPR, percentage of maximum possible response.

been extensively studied, most of those studies were based on dose-response relationships (Craft and Dykstra, 1990; Paronis and Holtzman, 1992), and, consequently, no data are available about the contribution of pharmacokinetic and pharmacodynamic (response versus drug concentration relationship) changes to the response versus time profile and/or in the dose-response relationships. The current study was design to characterize the disposition and response of an opioid in healthy and morphine treated rats that developed opioid dependence; such dependence was assessed by evaluating specific withdrawal signs. Methadone was used because it is the drug of choice in the treatment of withdrawal signs (Wolff et al., 1991) and because its *pk*/*pd* properties have been studied less than morphine (Gårdmark et al., 1993), alfentanil (Mandema and Wada, 1995), or buprenorphine (Ohtani et al., 1994). Methadone was administered by i.v. bolus to minimize the development of tolerance. The possible changes in the kinetic and dynamic processes were evaluated by the comparison of pharmacokinetic and pharmacodynamic parameters between healthy and abstinent rats. We used methadone plasma concentrations (total and unbound) as a measure of drug exposure, and the analgesic response recorded with tail-flick as the pharmacodynamic endpoint. The disposition of methadone in the brain was also evaluated.

Materials and Methods

This manuscript reports both in vivo and in vitro studies. Three in vivo experiments were performed: plasma (experiment I) and brain (experiment II) disposition of methadone and analgesic effect of methadone (experiment III). During the in vitro study, the plasma protein binding of methadone and the levels of α_1-acid glycoprotein (*AAG*) in plasma were determined. In each of these experiments, control and abstinent (showing withdrawal signs) rats were used. All experiments were carried out between 8:00 AM and 12:00 AM to minimize circadian variations.

In Vivo Studies

Animals and Surgical Procedure. Male Sprague-Dawley rats (220–280 g) obtained from Criffa (Barcelona, Spain) were kept at 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 4 days before the experiment was performed. The animals had free access to food and water. The protocol of the study was approved by the Committee on Animal Experimentation of University of the Basque Country.

Two minipumps (Alzet mod 2001, Palo Alto, CA) filled with saline (control group) or morphine solution (abstinent group) were implanted at 1cm under the skin of each animal. Under light ether anesthesia, the implant was inserted behind the neck next to the dorsal area; this area was shaved and swabbed with povidone iodide. The minipumps were removed under light ether anesthesia 6 days later and at the same time, a polyethylene catheter [inside diameter (I.D.) 0.5 mm and 10 cm long, Vygon, France] was implanted in the right jugular vein for the i.v. administration of methadone. In the animals from experiment I, the carotide artery was cannulated with a polyethylene catheter (I.D. 0.5 mm and 10 cm long, Vygon) for blood sample collection. All catheters were filled with a physiological saline solution containing 1% of heparin (50 I.U./ml; Roger Lab, Spain) to prevent clotting. The catheters were tunneled under the skin and externalized on the dorsal surface of the neck.

Induction of Physical Dependence on Morphine. Minipumps filled with saline morphine solution were used to induce physical dependence in treated animals. Morphine was delivered continuously at a rate of 0.5 μl/h for 6 days (Adams and Holtzman, 1990); the daily dose was 10 mg/kg.

Morphine Withdrawal and Abstinence Syndrome Measurement. The severity of spontaneous-abstinence syndrome was evaluated in control and morphine-treated rats just before (0 h) and 12 h after minipump removal. Each animal was placed in an acrylglass box and was observed for a period of 10 min. The following signs were measured: hostility on handling, diarrhea, and body weight loss. These signs are considered the most representative in the evaluation of abstinence syndrome (Bläsig et al., 1973). Diarrhea and hostility on handling were recorded as 0 (absent) or 1 (present). Body weight loss was evaluated as the difference between the weights recorded just before and 12 h after minipump removal.

Morphine Assay. In each animal, plasma levels of morphine were measured by RIA kit Coat-a-Count serum morphine (Dipesa, Spain) at 0 and 12 h after the minipumps were removed. A volume of 100 μl of blood was withdrawn through the catheter placed in the jugular vein. The blood was centrifuged at 2500 rpm for 15 min and 25 μl of plasma were used for morphine determination. This procedure was sensitive to 0.8 ng/ml and the antiserum had only 0.2% cross reactivity with morphine glucuronides (Bhargava et al., 1992).

Experiment I: Disposition of Methadone in Plasma. Twelve hours after the minipumps were removed, the control (n = 41) and abstinent (n = 33) animals, received a 0.35 mg/kg i.v. bolus dose of methadone in 30 s. The drug was administered in a saline solution. This dose of methadone was found to elicit analgesia with minor adverse respiratory effects (Gómez et al., 1995). Blood samples (one for each animal) were obtained at 1, 2, 3, 4, 5, 7.5, 10, 15, 30, 60, 90, and 120 min after drug administration; 3 to 4 animals were used at each time point. Arterial blood samples (2 ml) were centrifuged at 2500 rpm for 15 min. The plasma was immediately analyzed by high-performance liquid chromatography following the method of Wolff et al. (1991) with some modifications. Methadone and benzhexol (used as internal standard) were determined by UV detection using a wavelength of 250 nm. Afterward 0.5 ml of 1 M sodium carbonate buffer solution (adjusted to pH = 10 with sodium hydrogen carbonate solution 1 M) and 5 ml of a saturated water solution of n-butyl chloride were added to 1 ml of plasma in glass tubes. The mixture was mechanically shaken for 15 min and then centrifuged at 4°C for 10 min at 4000 rpm. The n-butyl chloride upper layer was placed in a 5-ml glass tube and then evaporated at 50°C (AES 1000, Speed Vac concentrator; Savant, Spain). The residue was dissolved in 0.1 ml of methanol, and a volume of 50 μl was injected into the chromatographic system. The liquid chromatographic system consisted of a pump (HPLC pump 422; Kontron Instruments, Bilbao, Spain), a UV detector (Waters 480), and an integrator (Y-549, Kontron Instruments, Bilbao, Spain). Chromatography was performed on a silica 5-μm column, Apex I 25- × 0.46-cm I.D. (Teknocroma, Barcelona) at 20°C. The mobile phase consisted of a mixture of methanol:1,2-dichloroethano:isopropanol 100 g/liter ammonium perchlorate aqueous solution (90.5:5:4:0.5 v/v) at a flow rate of 2 ml/min. This analytical procedure is sensitive to 5 ng/ml for methadone and linear over the range 5 to 250 ng/ml; the mean intra- and interassay precision and extraction efficiencies were 91.0% (5.6), 92.4% (3.2), and 87.1% (4.7) [mean (S.D.); n = 3], respectively.

Experiment II: Disposition of Methadone in Brain. Twelve hours after the minipumps were removed, the animals, control (n = 38) and abstinent (n = 37), received a 0.35 mg/kg i.v. bolus dose of radiolabeled methadone. The drug was administered in 30 sec. The animals were sacrificed by decapitation at 1, 2, 3, 4, 5, 7.5, 10, 15, 30, 60, 90, and 120 min after drug administration; 3 to 4 animals were used at each time point. The brains were rapidly removed and 100 mg of brain (cortex) was dissected (Dhawan et al., 1996). Tissue samples (100 mg) were mixed with 1 ml of tissue solvent (Scharlau, Barcelona, Spain) and were incubated for 48 h at 37°C; 10 ml of scintillation solution (NEF 989, DuPont, Barcelona, Spain) was added and the mixture was placed at room temperature for 2 h. The [^{14}C]methadone concentrations were measured by scintillation

counting using a Packard model 300-Tri-Carb Spectrometer (Gómez et al., 1995).

Experiment III: Analgesic Assay. The pharmacodynamics of methadone was studied in the control group (n = 18) and abstinent group (n = 15). The radiant-heat tail-flick technique was used to assess the nociceptive threshold (D'Amour and Smith, 1941). Tail-flick latency was measured automatically with a Letica (Barcelona, Spain) analgesimeter. The intensity of heat was adjusted so that control latencies were 2 to 3 s; animals with longer baseline latencies were excluded. A maximal cut-off time of 10 s was used to prevent tissue damage. The animals were tested for analgesia at 12 h after minipump removal (this value was taken as the baseline level for each animal) and at the following times after methadone administration: 1, 2, 3, 4, 5, 10, 15, 30, 45, 60, 90, and 120 min (control) and 1, 5, 7.5, 10, 15, 30, 45, 60, 90, and 120 min (abstinent). Antinociception was expressed as a percentage of maximum possible response (%MPR) and was calculated using the following expression:

%MPR = (test latency – baseline latency) * 100/(cut-off time – baseline latency)

In Vitro Studies

Animals and Surgical Procedure. Male Sprague-Dawley rats (220–280 g) obtained from Criffa (Barcelona, Spain) were kept in the same conditions as the animals used for in vivo studies. For the in vitro studies, two groups were also used, a control group (n = 10) and abstinent group (n = 10). To induce physical dependence on morphine, two minipumps filled with morphine solution or saline, were implanted in these animals. The surgical procedure was the same as described in the in vivo studies. No catheters were implanted in these groups of animals. The spontaneous abstinence syndrome was also assessed as was described above for the in vivo experiments. All animals were sacrificed by decapitation 12 h after minipump removal, and the blood was collected and immediately centrifuged for 15 min at 2500 rpm. Plasma samples (3 ml) were stored at –20°C for quantification of [^{14}C]methadone plasma protein binding, *AAG,* and morphine levels.

^{14}C-Methadone Plasma Protein Binding and Mucoprotein Assay. The protein binding was determined at 37°C by ultrafiltration, using a micropartition system (Amicon MPS-1) (March and Blanke, 1985). An aqueous solution (10 μl) of [^{14}C]methadone was added to 990 μl of plasma to a final drug concentration of 70 ng/ml (analgesic concentration in rats; Gómez et al., 1995). These samples were centrifuged at 3000 rpm for 5 min, and a volume of 100 μl was collected to measure the unbound methadone concentrations by scintillation spectrometry (Packard model 300-Tri-Carb Spectrometer). For mucoprotein assay, 0.5 ml of plasma were used to evaluate *AAG* levels, measured as mucoproteins, following Thaw and Albutt's method (Thaw and Albutt, 1980).

Data Analysis

Pharmacokinetic Analysis. The total concentration of methadone (C) and unbound plasma concentration of methadone (C_u) versus time profiles were described by a two-compartmental model. C_u in control and abstinent rats was obtained by multiplying C by the corresponding mean value of the unbound fraction in plasma (f_u) obtained from the in vitro studies. The total brain concentration of methadone (C_{brain}) versus time profiles were described by the following model:

$$dC_{brain}/dt = k_{13} \times C - k_{31} \times C_{brain}$$

where dC_{brain}/dt represents the rate of change of C_{brain}, k_{13}, and k_{31} are first-order rate constants governing the distribution processes of methadone between plasma and brain; for such analyses, the predicted C versus time profiles obtained from the previous pharmacokinetic analysis were used.

Estimation of Effect-Site Concentrations for Pharmacodynamic Modeling. Because a counterclockwise hysteresis was noted for both control and abstinent rats, when mean response data were plotted against predicted C in a time-ordered manner, a model linking the plasma compartment with the effect site by a first-order process, the exit rate for which is denoted by k_{e0}, was used; k_{e0} was estimated nonparametrically (Verotta and Sheiner, 1987). Once k_{e0} has been obtained, the effect-site concentrations are generated and can be used in the pharmacodynamic analyses.

Pharmacodynamic Analysis. The mean response versus predicted effect-site concentration relationships were described using the Sigmoidal E_{max} model.

All of the analyses were performed using the "naive-pool data" approach, in which all the data were fitted together as if they had come from a single individual. In attempting to propose a model with the maximum number of parameters in common between the control and abstinent rats, the data from the two groups were fitted simultaneously. In the first stage, a reduced model was fitted to the data; in this model, the two groups share all the parameters. In the following stage, the models fitted to the data have some parameters in common for the two groups, but not all; the last model fitted is the full model, which has a complete different set of parameters for each group. All the analyses were performed using the NONMEM (Beal and Sheiner, 1992) computer program. The model selection was based on a number of criteria, including 1) the visual inspection of the residual plots, 2) the confidence intervals of the parameter estimates, and 3) the value of the objective function given by NONMEM. The difference between the objective function values for two hierarchical models is approximately χ-square distributed and may be used for model selection (Boeckmann et al., 1992). In this study $P < 0.05$ was used as the level of significance.

Statistical Analysis. Comparisons between f_u and *AAG* levels from the two groups were made by the unpaired t test. Comparisons between weights and plasma levels of morphine just before and 12 h after minipump removal were made by the paired t test, while the differences in the hostility on handling and diarrhea between 0 and 12 h after minipump removal were evaluated by the paired McNemar test.

Drugs. Morphine clorhydrate and methadone clorhydrate were supplied by Alcaliber (Madrid, Spain). [^{14}C]methadone (specific activity 30 mCi/mmol and 98.8% of purity) was supplied by Amersham (Barcelona, Spain). Benzhexol clorhydrate was obtained from Lederle Lab. (Madrid, Spain). All reagents and solvents used were of analytical grade.

Results

Withdrawal and Morphine Measurements. Figure 1 shows the percentage of animals from the abstinent group displaying hostility on handling, diarrhea (top), and body weight loss (bottom) at 0 and 12 h after minipump removal. The percentage of animals showing hostility on handling and diarrhea after 12 h was statistically increased ($P < .0001$) with respect to the number of animals showing the same signs at 0 h; in addition, a significant decrease ($P < .0001$) in body weight was found between 0 h (286 ± 11 g) and 12 h after minipump removal (267 ± 10 g). Plasma levels of morphine at 12 h (14 ± 3 ng/ml) were found to be significantly decreased ($P < .0001$) with respect to those obtained at 0 h (111 ± 9 ng/ml). No significant differences in any of the withdrawal signs between 0 and 12 h after minipump removal were found in the control group.

Plasma Disposition of Methadone. C and C_u levels of methadone versus time profiles were best described, in both control and abstinent groups, by a two-compartmental model. Figures 2 and 3 show the experimental versus time data with the pharmacokinetic model predictions superimposed for C and C_u, respectively. Table 1 lists the estimates

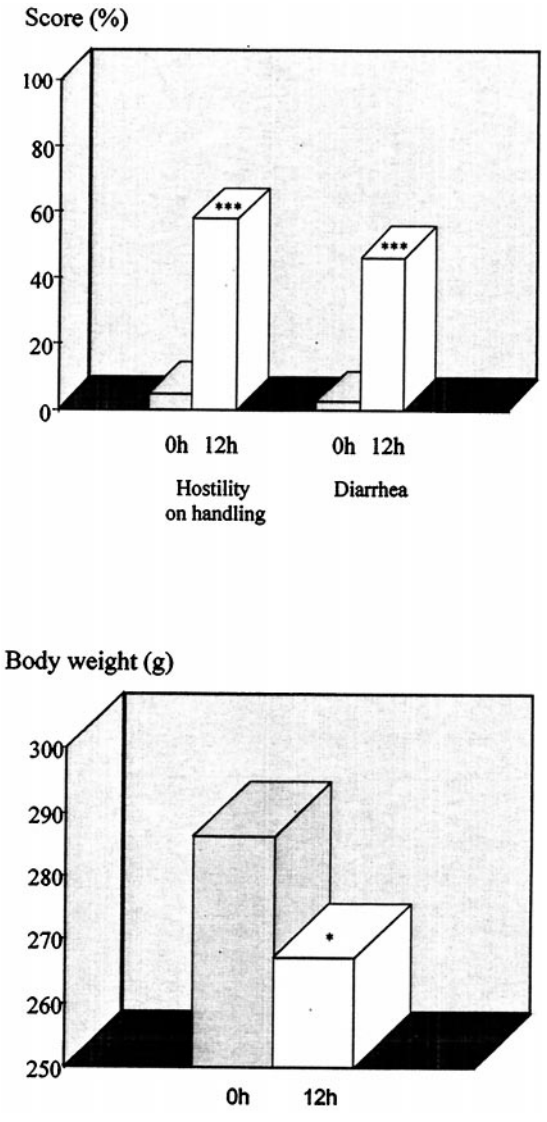

Fig. 1. Percentage of animals from abstinent group displaying hostility on handling and diarrhea at 0 h and 12 h after minipump removal (top); and the body weight loss of animals from abstinent group at 0 h and 12 h after minipump removal (bottom). *** ($P < .0001$), * ($P < .05$) statistically different from 0 h.

of the pharmacokinetic parameters and their coefficients of variation obtained during the analysis of the C versus time profiles. Both groups showed similar initial volume of distribution (V_1) and mean residence time (MRT) values; however, in abstinent rats, volume of distribution at steady state (V_{ss}), distribution clearance (Cl_d), and total plasma clearance (Cl) were 42%, 23%, and 40% decreased, respectively, in respect to the control group ($P < .05$). The in vitro binding of methadone to plasma proteins and the AAG levels were significantly increased ($P < .05$) in the abstinent group: f_u, 0.172 ± 0.1, abstinent, 0.254 ± 0.11, control; AAG, 1.30 ± 0.12 g/liter, abstinent, 0.36 ± 0.16 g/liter, control. Table 2 lists the estimates of the pharmacokinetic parameters and their coefficients of variation obtained during the analysis of the C_u versus time profiles. Both groups showed similar plasma unbound concentrations kinetics (see Fig. 3); estimates of unbound initial volume of distribution (V_{1u}), unbound distribution clearance (Cl_{du}), and unbound plasma clearance (Cl_u) did not differ between the two groups; only unbound volume of distribution at steady state (V_{ssu}), and unbound mean residence time (MRT_u) were 18% and 27% decreased, respectively, in abstinent rats with respect to the control group ($P < .05$).

Brain Disposition of Methadone Fig. 4 shows the C_{brain} versus time data for both control and abstinent rats and the predicted values obtained from the fit according to the model described in the methods section. Estimates of brain disposition parameters, and their coefficients of variation were the following: k_{13}= 2.1(15), control; 3.2 (20) min^{-1}, abstinent ($P < .05$); k_{31}= 0.30 (7), control; 1 (10) min^{-1}, abstinent ($P < .05$). The plasma to brain distribution clearance (Cl_{db}) and the apparent volume of the brain compartment (V_T) were determined using the following expression:

$$Cl_{db} = k_{13} \times V_1 = k_{31} \times V_T$$

Using the estimates of V_1 listed in Table 1, Cl_{db} was estimated to be 2.50, control, and 4.05 liters/min/kg, abstinent; V_T was estimated in 8.3, control and 4.05 liters/kg abstinent. Based on model predictions, the maximums were achieved at 10 min after drug administration in the control group and almost immediately after drug injection in the abstinent group.

Pharmacodynamics of Methadone. Figure 5 shows the time course of the mean analgesic effect in both groups. Analgesia was significantly ($P < .05$) higher in control animals at times 1, 5, 10, and 15 min after drug injection. Baseline analgesic values did not differ between the two groups. In both groups the mean maximum of analgesia appears at approximately 10 min after methadone administration. When the analgesic effect versus C data were plotted in a time-ordered manner, counterclockwise hysteresis was noted; the greatest effect was seen after the peak plasma concentrations. Table 3 lists the pharmacodynamic parameters and their coefficients of variations. E_{max} (maximum attainable effect) was 65% decreased ($P < .05$), and C_{50} (steady-state plasma concentration of methadone eliciting 50% of E_{max}) was 38% increased ($P < .05$) in the abstinent group in respect to the control group; k_{e0} (rate constant of equilibrium between the effect and plasma compartments), was 28% increased in the abstinent rats. The half-life of equilibration between plasma and effect-site compartment was 0.67 min and 0.48 min, for abstinent and control group, respectively. Figure 6 shows the analgesic effect versus estimated effect-site concentration relationships for both groups. This relationship was best described in both groups by a sigmoidal E_{max} model. When C_{50} estimates were corrected by the differences in the plasma protein binding found between two groups, the unbound C_{50} values were 6.14, control, and 5.78 ng/ml, abstinent. Figure 7 shows the time course of the mean observed and the model predicted (based on total plasma concentrations) analgesic effects.

Discussion

In this article, we have explored the changes in the plasma and brain disposition kinetics in addition to the changes in the pharmacodynamic relationships of methadone occurring during withdrawal from morphine in the rat.

The method used in the current study to induce opioid

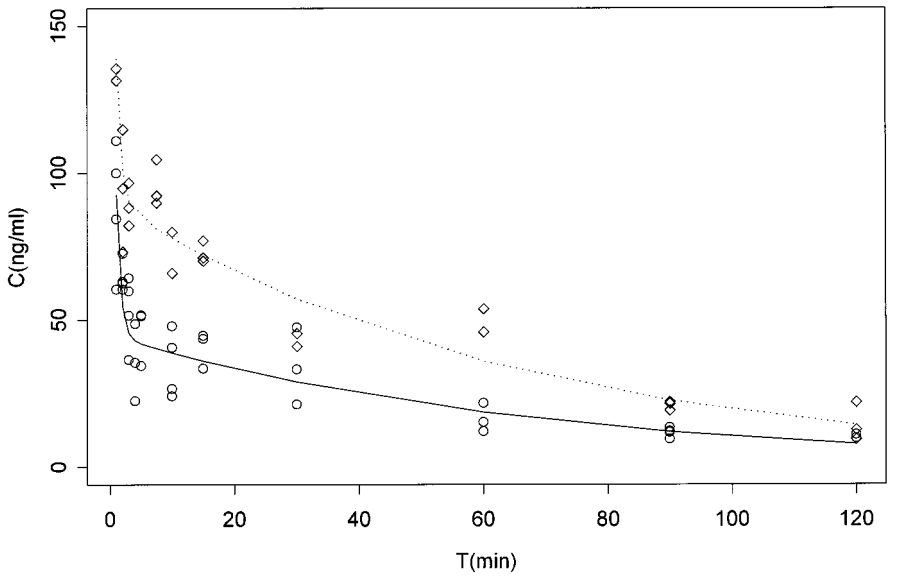

Fig. 2. Total plasma concentrations of methadone versus time profiles after a 0.35 mg/kg i.v. bolus dose. Symbols represent experimental observations: open circles (control), diamonds (abstinent). Lines represent model predictions: solid (control), dashed (abstinent).

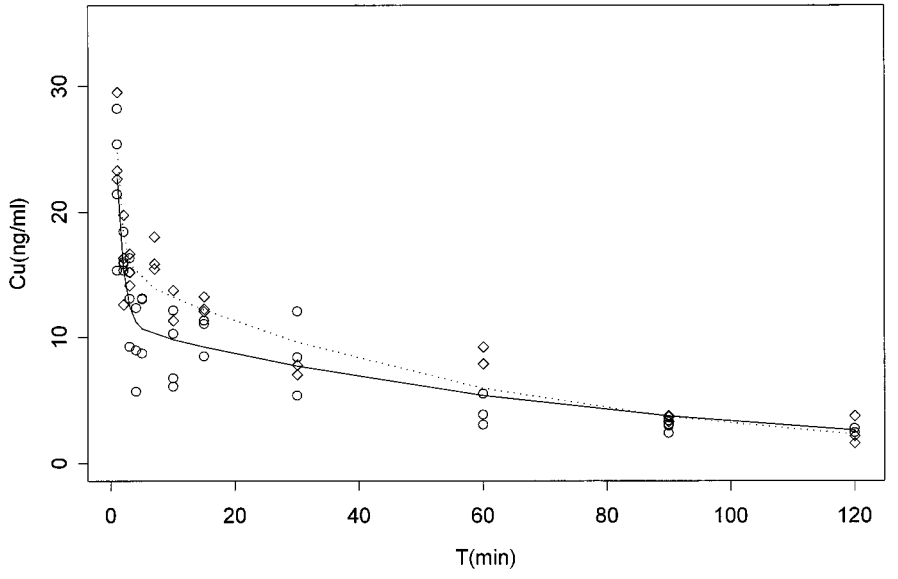

Fig. 3. Unbound plasma concentrations of methadone versus time profiles after a 0.35 mg/kg i.v. bolus dose. Symbols represent experimental observations: open circles (control), diamonds (abstinent). Lines represent model predictions: solid (control), dashed (abstinent).

TABLE 1

Unbound fraction in plasma [mean (CV(%)] and pharmacokinetic parameters based on total concentrations of methadone in plasma expressed as estimate [CV(%)] in control and abstinent rats after a 0.35 mg/kg i.v. bolus dose

Parameter	Control	Abstinent
f_u	0.254 (45)	0.172 (61)*
V_1 (l/kg)	1.24 (34)	1.35 (34)
V_{ss} (l/kg)	6.55 (9)	3.82 (6)*
Cl_d (l/min/kg)	1.45 (10)	1.12 (22)*
Cl (l/min/kg)	0.10 (17)	0.06 (10)*
MRT (min)	64.7 (45)	62.9 (12)

* Different from control group ($P < 0.05$).

TABLE 2

Pharmacokinetic parameters based on unbound concentrations of methadone in plasma expressed as estimate [CV(%)] in control and abstinent rats after a 0.35 mg/kg i.v. bolus dose

Parameter	Control	Abstinent
V_{1u} (l/kg)	7.75 (34)	8.42 (34)
V_{ssu} (l/kg)	27.3 (9)	22.5 (6)*
Cl_{du} (l/min/kg)	5.10 (10)	5.54 (22)
Cl_u (l/min/kg)	0.34 (17)	0.36 (10)
MRT_u (min)	80.3 (417)	60.9 (16)*

* Different from control group ($P < 0.05$).

dependence has been previously used by other investigators (Adams and Holtzman, 1990). This method, which involves the continuous exposure to morphine for 6 days, has been found suitable for producing physical dependence and cross-tolerance. The degree of physical dependence was assessed by emergence of a spontaneous withdrawal syndrome after removal of the maintenance drug. Many investigators have characterized this phenomenon by measuring some behavioral or/and physiological changes, such as weight loss, diarrhea, and hostility on handling (Pierce et al., 1996). On the basis of the results obtained for those signs (Fig. 1), it can be concluded that the rats receiving morphine continuously for 6 days presented a clear abstinence syndrome 12 h after minipump removal.

Our results obtained from the in vitro experiments showed a significant increase in the levels of *AAG* in the abstinent group. That increase was similar to the one reported by

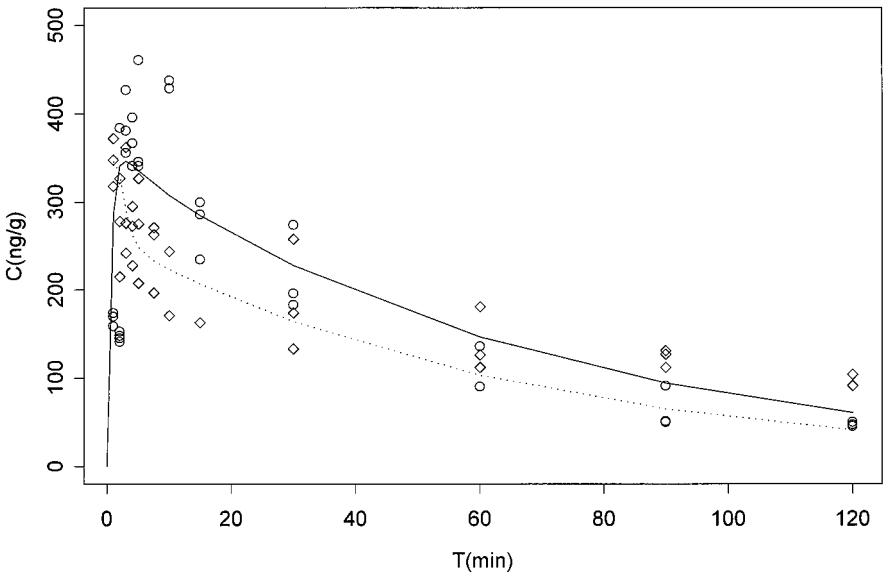

Fig. 4. Brain concentrations of methadone versus time profiles after a 0.35 mg/kg i.v. bolus dose. Symbols represent experimental observations: open circles (control), diamonds (abstinent). Lines represent model predictions: solid (control), dashed (abstinent).

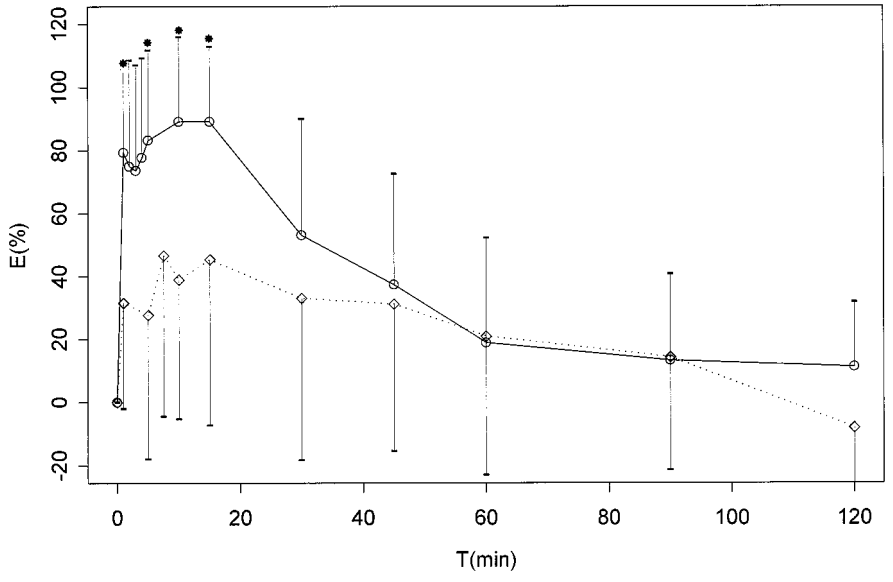

Fig. 5. Time course of the analgesic effects of methadone after a 0.35 mg/kg i.v. bolus dose. Symbols represent mean experimental observations: open circles (control), diamonds (abstinent). Lines represent linear interpolation: solid (control), dashed (abstinent). Vertical lines represent standard deviations: * statistically different from abstinent group ($P < .05$).

TABLE 3
Pharmacodynamic parameters expressed as estimate [CV(%)] in control and abstinent rats after a 0.35 mg/kg i.v. bolus dose

Parameter	Control	Abstinent
E_{max} (%)	85.8 (6.2)	38.7 (14)*
C_{50} (ng/ml)	24.2 (6.6)	33.6 (14)*
γ	4.8 (25)	4.8 (25)

* Different from control group ($P < 0.05$).

Garrido et al. (1996) 12 h after morphine withdrawal in the rat. There are many physiopathogical alterations in which the *AAG* levels are significantly increased (burns, surgery, inflammatory diseases; Gómez et al., 1995); in general all those conditions are stress related. Withdrawal signs, such as weight loss, hostility on handling, and diarrhea, can be considered reactions to a high-stress situation, leading therefore to an increase in the *AAG* levels. Similar results were obtained in a study comparing the *AAG* levels between healthy volunteers and opioid addict subjects (unpublished results).

Methadone binds predominantly in plasma to *AAG* to a high degree, 86 to 89%, in humans (Inturrisi et al., 1987) and 80% in the rat (Gómez et al., 1995). These protein binding characteristics could explain the decrease in f_u in plasma found in the abstinent group with respect to control rats. There is also the possibility of a protein binding interaction between methadone and morphine and/or metabolites of morphine; but given the low protein binding characteristics of morphine and metabolites (Vozeh et al., 1989), such interaction is unlikely to occur. In addition, the value of f_u obtained for the abstinent group is in agreement with the value of f_u in plasma reported from animals suffering an induced experimental inflammation with a similar increase in the *AAG* levels to that observed in the current study (Gómez et al., 1995).

The estimates of the parameters obtained in the current study are in accordance with those published previously by Ling et al. (1981) in control rats and by Gabrielsson et al. (1985) in a study carried out in pregnant rats. However, significant changes between the abstinent and control rats were found in the plasma disposition of total concentrations

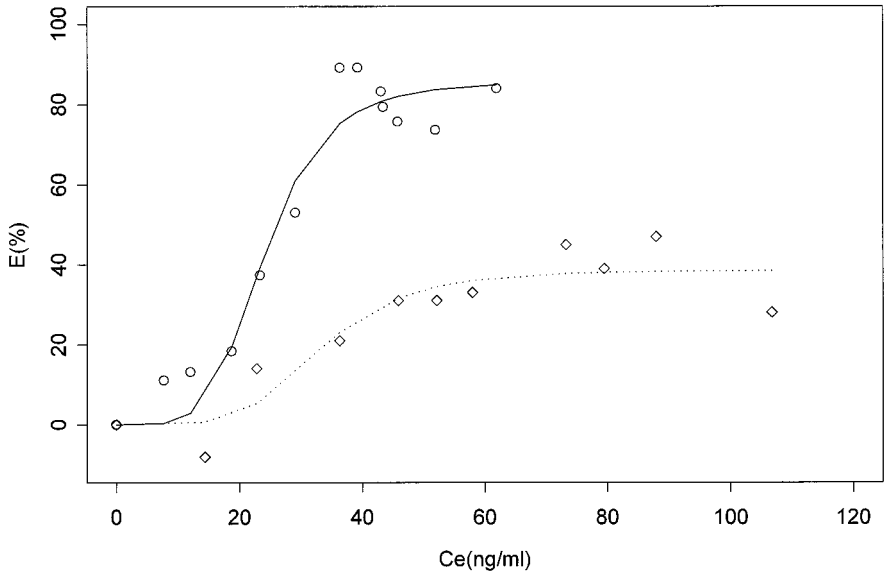

Fig. 6. Analgesic effect versus the effect-site concentration (C_e) relationship of methadone. C_e estimated based on total methadone plasma concentrations. Symbols represent mean experimental observations: open circles (control), diamonds (abstinent). Lines represent model predictions: solid (control), dashed (abstinent).

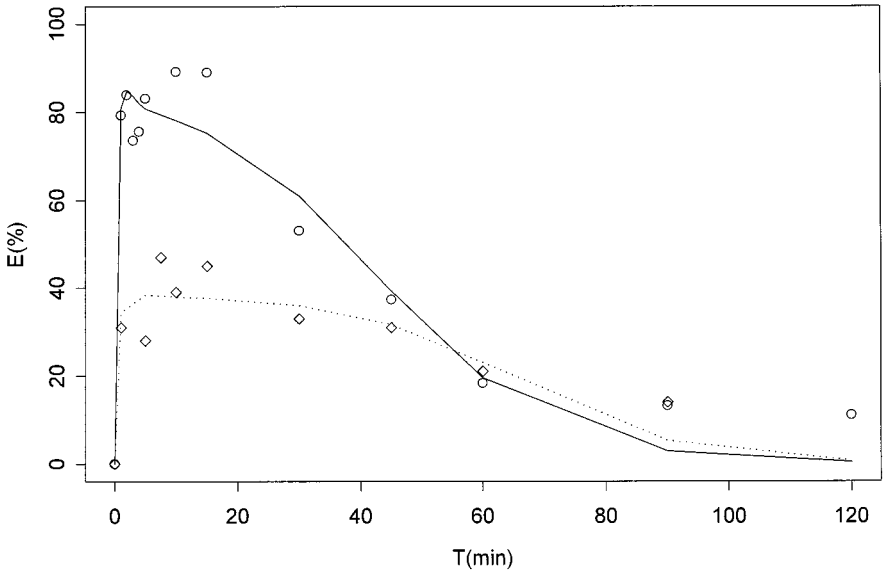

Fig. 7. Time course of the analgesic effects of methadone after a 0.35 mg/kg i.v. dose. Symbols represent mean experimental observations: open circles (control), diamonds (abstinent). Lines represent model predictions obtained from the pharmacokinetic/pharmacodynamic model selected: solid (control), dashed (abstinent).

of methadone. The changes were found in both the distribution and elimination processes. Given the parameter values obtained for the control group, methadone is an extensively distributed drug with a high plasma clearance, which a priori allows us to predict a proportional change in the apparent volume of distribution with a change in f_u in plasma (Rowland and Tozer, 1995a). Such a prediction seems to be confirmed by the results in Table 1. On the contrary, changes in f_u should not affect the total plasma clearance (Rowland and Tozer, 1995b). The decrease in the total plasma clearance found in the abstinent group is a result that is difficult to explain. We have no evidence of an eventual decrease in hepatic activity during withdrawal, and therefore we expected minor modifications in the clearance of methadone. Several hypotheses can be formulated, such as hepatic inhibition caused by continuous and previous exposure to morphine. Another possibility is related to the fact that the elimination of drugs can be affected by plasma proteins beyond simply regulating the f_u in plasma.

Results from several studies have suggested a decrease in the intrinsic clearance of drugs in situations in which the *AAG* levels were increased (Quin et al., 1994). The estimates of the pharmacokinetic parameters based on unbound plasma concentrations showed that most of the changes found in the plasma disposition of total concentrations of methadone in the abstinent group could be explained by the decrease in f_u (see Fig. 3).

We measured [^{14}C]methadone in the brain because in previous studies it has been demonstrated that more than 90% of [^{14}C]methadone in brain was unchanged (Liu et al., 1983). Disposition of methadone in the brain differs also between control and abstinent rats. Distribution of methadone in the brain in the abstinent group is faster (Fig. 4). This phenomenon could be explained by taking into account the fact that for highly lipophylic drugs, such as methadone, the rate of tissue distribution is a nonrestrictive process that depends on the total drug plasma concentrations rather that on the unbound plasma concentrations (Rowland and Tozer, 1995a). So, the higher total plasma concentrations found in the abstinent group produced a faster entry into the brain, as has

been reflected in the estimates of k_{13}. Similar results have been reported for other lipophylic drugs highly bound to *AAG*, such as lidocaine (Marathe et al., 1991), in situations in which the *AAG* levels were significantly increased. Several studies have explored the brain disposition of methadone in rats (Liu et al., 1983; Gabrielsson et al., 1985) but none compared control with withdrawal condition. Our results obtained from the control group are similar to those reported by Gabrielsson et al. (1985), where the partition coefficient between brain and plasma was estimated as 4.57, a value similar to the one estimated in the present study using the Chen and Gross approach (Chen and Gross, 1979).

Figure 5 shows a significant decrease in the analgesic effect versus time profile for the abstinent group with respect to the control group. One might think, on the basis of our results regarding the decrease in the extension of drug distribution, that the observed minor effect could be caused by kinetic modifications. To analyze eventual alterations in the dynamic processes, pharmacodynamic modeling of the data is required. Because in the current study we measured total plasma and brain methadone concentrations, we needed to choose the most adequate drug exposure measurement before data modeling was possible. We decided to use the total plasma concentrations for three reasons: 1) brain concentrations are rarely measured, although, methadone plasma concentrations have been measured in patients in several studies (Inturrisi et al., 1987); 2) as can be observed from Figs. 4 and 5, the brain methadone concentrations versus time profile in the case of control group appears to be in equilibrium with the analgesic effect versus time profile, however, a counterclockwise hysteresis was noted for the abstinent group; and 3) there are alternatives, like the effect compartment model (Sheiner et al., 1979), that allows for pharmacodynamic parameter estimations in cases of temporal displacements between plasma drug concentration and response.

The value of k_{eo} obtained for the abstinent rats was estimated as being lower than the one obtained for the control rats. For the morphine-treated group, taking into account the fact that entry into the brain is almost instantaneous after drug injection, the value of k_{eo} would represent delays in signal transduction; but for the control group, k_{eo} could reflect the delay in entering into the brain. To analyze the dynamic properties of methadone, we simply related the mean observed responses with the estimates of the methadone effect-site concentrations using a sigmoidal E_{max} model. Tolerance development has been described after bolus injection of morphine (Kissin et al., 1991); if such acute tolerance development had occurred in the current study, a clockwise hysteresis would be noted in the effect versus plasma concentration plot. No such phenomenon was observed, and we concluded that either no or minor acute tolerance for methadone was developed. There is a possibility of a pharmacodynamic drug interaction between methadone and morphine and/or metabolites of morphine in the abstinent group, but we think that such interaction is unlikely because the measured morphine plasma concentrations are very low in comparison with the values of C_{50} for morphine estimated by other authors [approximately 400 ng/ml by Ouellet and Pollack (1997)] and also because the baseline analgesic values obtained were not significantly different from those obtained for the control group.

Another important issue in the pharmacodynamic analysis is the presence of active metabolites that can hamper the interpretation of results. The data in the literature suggest that the main metabolites of methadone are pharmacologically inactive (Liu et al., 1983). Methadone is commonly administered as a racemic mixture of the *l*- and *d*-isomers, the *l*-isomer being responsible for the opioid pharmacological activity and the *d*-isomer inactive. Recently, Kristiensen et al. (1996) reported the pharmacokinetic parameter estimates for both isomers in humans. The estimates for clearance were 156 and 130 ml/h for the *l*- and *d*- isomers, respectively. The values of the apparent volume of distribution at steady state were 490 (*l*-isomer) and 290 (*d*-isomer) liters. Both isomers showed a similar high value for plasma protein binding (approximately 90%). These data allow us to assume a parallel shift in the kinetics of both isomers during withdrawal with respect to the kinetics in a control condition. On the basis of the above, the use of a simple model to relate response with effect site drug concentrations seems to be appropriate.

The estimate of drug potency (C_{50}) was 24.2 ng/ml. That estimate, when corrected by f_u in plasma, was 6 ng/ml (1.6 nM), a value close to the one reported by Magnan et al. (1982) for the affinity of methadone for the μ-opioid receptors (K_I = 4.5 nM). Once C_{50} for both groups was corrected by the changes in f_u, drug efficacy reflected by the estimate of E_{max} was the only pharmacodynamic parameter differing between the two groups. There is no clear consensus about what type of alteration in the μ-receptor system is involved by previous exposure to opioids or/and during an abstinence syndrome. Several investigators have reported changes at the receptor level, in terms of down-regulation (Díaz et al., 1995) after continuous exposure to an opioid, but also an up-regulation of the opioid system between 6 to 72 h after a spontaneous abstinence syndrome of morphine, (Ullibarri et al., 1987; Bhargava and Gulati, 1990). We cannot think of a mechanism that could explain the results of our study in terms of up-regulation of μ-opioid receptors. In contrast, the decreased intrinsic activity of methadone after chronic treatment with morphine is consistent with expectations for down-regulation when the receptor reserve is limited. On the other hand, the decrease in the E_{max} could be explained by changes in the number of receptors and also by changes at the postreceptor level such as transducer molecules (Kramer et al., 1997). The fact that the C_{50} values based on unbound concentrations were not different between the two groups leads us to assume that alterations related to the sign transduction processes account for most of the changes in E_{max}.

Paronis and Holtzman (1992) studied the development of tolerance to the analgesic activity of several opioids including methadone after continuous infusion of morphine, meperidine or fentanyl in rats. They found that after previous exposure to morphine, the estimates of ED_{50} of methadone were not significantly different from those obtained after a saline infusion. The fact that they compared ED_{50} instead of C_{50}, and that they administered methadone at 24 h after minipump removal instead of 12 h, as in our study, could play a role in the differences seen between both studies.

To summarize the results of the current study, spontaneous withdrawal was associated with increasing levels of α_1-acid glycoprotein levels in plasma and a decrease in f_u in plasma. The altered protein binding accounted for most of the changes found in the plasma and brain disposition of methadone. Pharmacodynamic changes reflected by a decrease in

the maximum attainable analgesic effect were seen after withdrawal; this result together with the absence of alterations in C_{50} supports the hypothesis of a change at the postreceptor level. On the basis of the results reported in the current study the altered effect versus time profiles found during withdrawal are mediated mostly by pharmacodynamic changes.

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