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Influence of Plasma-protein Binding on Analgesic Effect of Methadone in Rats with Spontaneous Withdrawal

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Abstract

The effect of spontaneous withdrawal on α_1-acid glycoprotein (AAG) levels and methadone protein binding has been studied in the rat.

Animals were made physically dependent on morphine by providing morphine HCl in drinking water for three weeks. The natural opiate withdrawal was induced in rats by substituting the morphine solution with drinking water. The severity of the abstinence syndrome was assessed at various time intervals. After 12 h of withdrawal, the animals showing abstinence signs and low morphine levels were injected with intravenous methadone (0·35 mg kg^{-1}) and the analgesic effect was measured by the tail-flick method and compared with animals receiving water.

The oral administration of morphine produced an increase in AAG levels from 0·64 ± 0·05 g L^{-1} in control animals to 1·47 ± 0·92 g L^{-1} in experimental animals at the point of withdrawal and 1·21 ± 0·09 g L^{-1} 24 h after withdrawal. The percentage of methadone unbound was significantly lower in morphine-treated than in control animals. A significant correlation between AAG levels and percentage of methadone bound was observed. A parallel analgesic effect after intravenous methadone, as measured by AUC in the tail-flick test, was less in abstinence animals than in control (287·6 ± 24·8 compared with 401·0 ± 37·06 s min). We suggest that in the withdrawal syndrome an adjustment of methadone dose may be necessary because of changes in protein binding.

The abstinence syndrome has been associated with hyperactivity of the central noradrenergic neurons indicating a correlation between the severity of opiate withdrawal syndrome and marked increase in the release of noradrenaline (Swann et al 1983). In heroin addicts, biochemical and functional evidence of α_2-adrenoceptor supersensitivity in blood platelets has been demonstrated during withdrawal. A correlation between the density of platelet α_2-adrenoceptors and the severity of heroin withdrawal has been observed (García-Sevilla et al 1985, 1987). The inhibitory α_2-adrenoceptors could be involved in the regulation of release of noradrenaline during reaction to opiate withdrawal. Also modulation of brain α_2-adrenoceptor regulation has been demonstrated during the spontaneous withdrawal syndrome in the rat (Ullibarri et al 1987).

α_2-Adrenoceptors are glycoproteins and thus, a possible parallelism between an increase of α_2-adrenoceptors and other serum glycoproteins could be postulated. α_1-Acid glycoprotein (AAG) is the protein mainly involved in the binding of basic drugs (Routledge 1986). Several disease states are associated with elevated levels of this protein (Abramson 1982). A reduced analgesic response to mianserin and methadone has been observed in animals when an increase in AAG levels was experimentaly produced (Torres et al 1992; Gómez et al 1995).

Methadone is a basic drug which shows elevated protein binding to AAG. It is widely used in maintenance and detoxification of heroin adicts. Evidence indicates that there is a wide range of interindividual variability in methadone response, even among patients on fixed dosage schedules (Bell et al 1988; Loimer & Schmid 1992).

Therefore, in the present work, we evaluated in an animal model, whether an increase in AAG levels and consequently in methadone binding could be observed during spontaneous withdrawal syndrome and whether alteration of the analgesic effect could be associated with these protein-binding changes.

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Materials and Methods

Animals

Male Sprague–Dawley rats (140–160 g at the start of experiments) (n = 62) were used. The animals received a standard diet with water freely available and were housed at 20 ± 2°C with a 12 h light/dark cycle.

The animals were separated into two groups: control group (n = 22) and treated group (n = 40). The control group only took water and the treated group took the morphine in drinking water. These groups were used to carry out in-vivo and in-vitro studies.

Twelve control animals were used to study plasma-protein binding in-vitro of [^{14}C]methadone and another ten different animals to evaluate in-vivo the methadone analgesic effect.

Twenty-seven treated animals were used to measure morphine levels at different times after morphine withdrawal, nineteen out of those twenty-seven were also used to study the [^{14}C]methadone plasma-protein binding in-vitro. Another thirteen morphine-treated animals were used to evaluate the analgesic effect of methadone.

Induction of morphine dependence

The animals (5 rats per cage; 40 in total) were made physically dependent on morphine by the method of Badawy et al (1982). Morphine hydrochloride was provided in freely available drinking water as the only source of fluid. The drug was given in increasing concentrations (48 h apart) of 0·1, 0·15, 0·2, 0·3, 0·4, 0·5, 0·6, 0·8, 1·0 and 1·2 mg mL^{-1} (expressed as the salt), which represented morphine doses from 12 to 130 mg kg^{-1} per day during the 3-week induction period.

Abstinence syndrome measurement and withdrawal

Opiate withdrawal was induced in rats (n = 40) by substituting the morphine solution with drinking water. In three rats the withdrawal was induced by naloxone injection (1 mg kg^{-1}, i.p.).

The severity of the abstinence syndrome was assessed at various time intervals (0, 6, 12, 24, 48, 72 h) by quantifying the behavioural syndrome as described by Bläsig et al (1973) and Swann et al (1983). At each time interval after spontaneous morphine withdrawal, various withdrawal signs were checked (abnormal posturing, vocalization on touch, ptosis, diarrhoea, penile erection) and counted (rearing, jumping, wet-dog shakes, teeth chattering) when present during a 10-min period. For each rat the score on the withdrawal syndrome rating scale was the number of "checked" signs plus the number of times "counted" behaviours occurred during the observation period.

Morphine assays

Plasma samples from abstinence animals (n = 27) were used to measure morphine levels by the RIA kit "Coat-a-Count Serum Morphine" (Dipesa, Spain). The concentration of morphine was determined by RIA using 25-μL serum samples; this procedure could detect morphine down to 0·8 ng mL^{-1} and the antiserum had only 0·2% cross reactivity with morphine glucuronides (Bhargava et al 1992).

[^{14}C]Methadone-binding assays

Control (n = 12) and the animals with spontaneous withdrawal (n = 19) were killed by decapitation at different times during the abstinence period (0, 6, 12 and 24 h), and blood samples were collected and immediately centrifuged for 15 min at 2500 rev min^{-1}. Plasma samples were stored at −20°C for protein binding and mucoprotein assays.

In plasma from animals, [^{14}C]methadone binding was determined at 37°C by ultrafiltration, using a micropartition system (Amicon MPS-1) (March & Blanke 1985). An aqueous solution (10 μL) of [^{14}C]methadone was added to 1 mL plasma to a final concentration of 70 ng mL^{-1} (the therapeutic dose in rats); this concentration is close to the range of therapeutic levels in man (ca. 100 ng mL^{-1} according to Bell et al (1988) and Loimer & Schmid (1992)). Samples were centrifuged at 3000 rev min^{-1} for 5 min. Aliquots of 100 μL were collected and [^{14}C]methadone free concentration was measured by scintillation counting using a Packard model 300-Tri-Carb Spectrometer.

Mucoprotein assay

Plasma samples from all animals (control, after naloxone and spontaneous) were used to evaluate AAG concentration measured as mucoprotein (Thaw & Albutt 1980).

The tail-flick assay

For this study two groups of rats were used, a control group (water, n = 10) and a group of morphine-dependent rats (n = 13), which developed the spontaneous abstinence syndrome. After 12 h of withdrawal, the animals were injected intravenously with methadone (0·35 mg kg^{-1}) to assess the analgesic effect of methadone.

Methadone analgesia was measured by the tail-flick method. Tail-flick latencies to thermal stimulation were determined before and at various times up to 240 min after a single dose of methadone. Basal latencies were approximately 4 s. A cut-off time of 10 s was used to prevent any injury to the tail (D'Amour & Smith 1941).

A time–response curve was constructed for each rat; the area under the time–response curve ($AUC_{0\text{-}240}$) was individually calculated. The mean area under the time–response curve was calculated by a log-linear trapezoidal method.

Drugs

[^{14}C]Methadone (sp. act. 30 mCi $mmol^{-1}$) from Amersham (Spain) was used in the in-vitro studies and morphine HCl, methadone HCl (Alcaliber, Madrid, Spain) and naloxone HCl (Sigma, Spain) to inject the rats. The RIA kit "Coat-a-Count Serum Morphine" was from Dipesa (Spain).

Statistics

Results are expressed as mean ± s.e.m. Student's *t*-test was used in the comparison between groups. Linear regression analyses were carried out by Pearson's coefficient. Behavioural data (non-parametric) were analysed by the Mann–Whitney U-test. The level of significance was chosen as $P < 0.05$.

Results

The evolution of the time course for the severity of the spontaneous abstinence syndrome is shown in Fig. 1; the maximal score was seen at 24 h ($P < 0.0001$) when it was compared with the control group. Fig. 2 shows the morphine levels measured at different times of withdrawal.

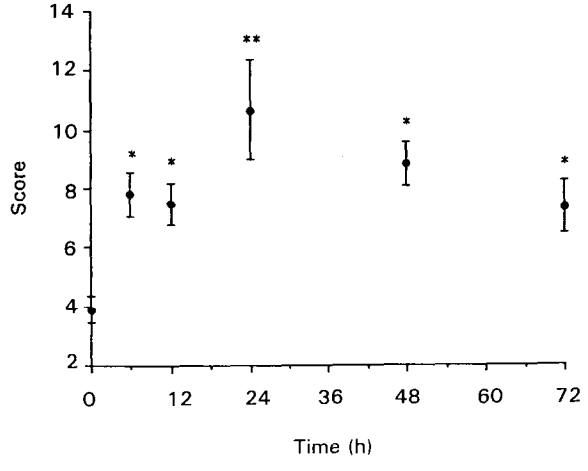

FIG. 1. Time course for the severity of the abstinence syndrome in rats after morphine withdrawal. The major score was at 24 h (**$P < 0.0001$) vs control group. *$P < 0.001$.

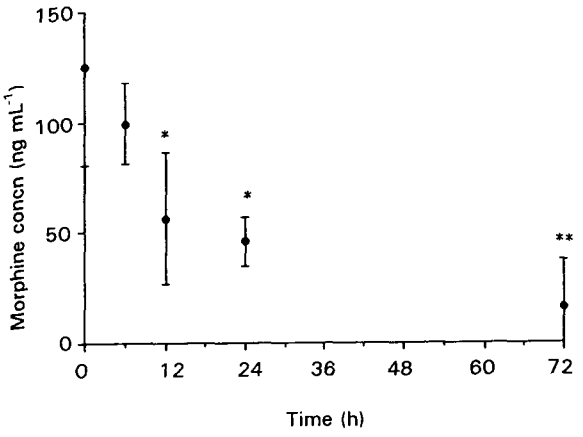

FIG. 2. Time course for the morphine levels during abstinence syndrome ($n = 27$). The levels at 12 h after morphine withdrawal were significantly minor (*$P < 0.001$) compared with that at 0 h. **$P < 0.0001$.

Morphine levels were significantly minor at 12 h ($P < 0.001$) after morphine withdrawal when compared with levels at 0 h. Therefore, we considered that this time could be appropriate to study methadone kinetics. The correlation between morphine levels and abstinence score was significant ($P < 0.05$).

The oral adminstration of morphine (21 days) led to an increase in AAG levels immediately after morphine solution withdrawal. These levels were similar during spontaneous abstinence from 0 to 24 h and were always statistically significant ($P < 0.0001$) when compared with controls (Table 1). After naloxone administration the AAG levels were of the same order as AAG levels obtained during spontaneous abstinence.

The results from the in-vitro study are shown in Table 1 and Fig. 3.

The percentage of methadone bound was significantly greater in abstinence than in control animals ($P < 0.0001$). A significant correlation ($P < 0.0001$) between AAG levels and percentage of methadone unbound was observed in all animals.

On the other hand, the methadone analgesic effect evaluated in-vivo was lower in abstinence animals than in control rats ($P < 0.05$); these results were expressed as area under the curve of analgesic effect–time from 0 to 240 min (AUC_{0-240}) in both groups (Table 2). The AAG levels in the two groups were in the same range as found in Table 1, for control and abstinence groups.

Discussion

It is a common feature for patients in methadone maintenance programs to complain that their dosage seems inadequate. However, it is difficult constructively to answer requests for increasing doses because there are no objective criteria by which to judge the adequacy of an individual dose (Bell et al 1988).

Some methadone pharmacokinetic changes can be responsible for low response after standard doses, and studies of the pharmacokinetics of methadone in patients maintained on the drug have suggested that there was a wide range of interindividual (and intra-individual) variability in characteristics such as the elimination half-life and clearance (Wolff et al 1993). Although plasma-protein binding could contribute to pharmacokinetic and pharmacodynamic variability, there is a lack of studies about protein binding of methadone in withdrawal individuals.

Methadone is a basic drug which is highly bound to AAG. There is a wide range of changes in the levels of this plasma protein between individuals (0·5–1 g L^{-1} in healthy volunteers) and it has been postulated as responsible for inter-individual variation in drug response of basic drugs.

High levels of AAG have been observed in cancer and in inflammatory diseases (Abramson 1982). In rats with experimental inflammation and with high AAG levels, decreased responses to methadone when compared with control have been observed. These changes were associated with an increase in methadone protein binding (Gómez et al 1995).

The present work clearly shows that natural opiate withdrawal, induced by substituting the morphine solution with drinking water, is associated with an increase in AAG levels when compared with rats before morphine intake. This increase was observed from 0 time to 24 h after substituting morphine solution, indicating that a degree of stress could have taken place during the development of morphine dependence. In similar experimental conditions an increase in the density of brain α_2-adrenoceptors was found in parallel with the intensity of the morphine abstinence syndrome. No changes in the μ-receptors were found in this situation (Ullibarri et al 1987).

On the other hand, withdrawal induced by naloxone did not produce changes in brain adrenoceptor density, whereas an increase of AAG has been observed by us in similar conditions. Production of AAG may be more rapid than modulation of hypothalamic α_2-adrenoceptors.

The extent of protein binding of methadone was different in morphine-treated animals immediately after withdrawal (time 0) when compared with control animals. Similarly,

Table 1. AAG levels and in-vitro protein binding of methadone (70 ng mL^{-1}) in control group (n = 12) and abstinent animal groups (from 0 to 24 h; n = 19).

	Control	Withdrawal			
		0	6	12	24
AAG (g L^{-1})	0·64 ± 0·05	1·47 ± 0·92*	1·38 ± 0·04*	1·42 ± 0·16*	1·21 ± 0·09*
Unbound (%)	16·98 ± 1·98	6·96 ± 0·47*	6·84 ± 1·14*	6·94 ± 0·46*	7·25 ± 1·56*

*$P < 0.0001$ compared with control.

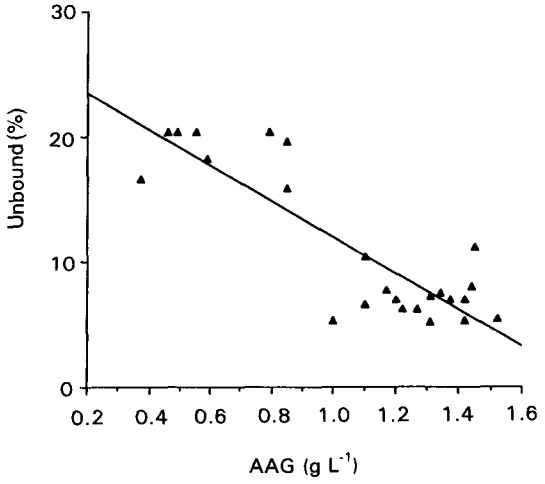

FIG. 3. Correlation between AAG levels and percentage of methadone unbound in the same animals. This correlation (r = 0·85) was significant with $P < 0.0001$.

an increase in plasma-protein binding of methadone in the abstinence group (time from 6 to 24 h) was observed. A significant correlation was seen between AAG levels and plasma-protein binding of methadone.

The decreased response observed to methadone in morphine abstinence compared with control rats established that in the withdrawal syndrome, an increase in methadone doses could be necessary.

Pharmacokinetic tolerance to methadone analgesia in rats implanted with morphine pellets has been reported (Liu & Wang 1985) using the radiant-heat tail-flick method of determining analgesia. Liu & Wang (1985) observed a decrease in the brain concentrations of methadone measured at 60 and 120 min. No change in the biotransformation of methadone was observed and, therefore, they suggested that the cross-tolerance to methadone analgesia seen in chronic morphine-implanted rats was partly associated with a decrease in the brain concentration of methadone occurring by a mechanism not directly related to a change in the biotransformation of methadone. They conclude that changes in the distribution of methadone in the brain could explain their observations.

The results obtained in the present work support this hypothesis because an increase in protein binding can limit the drug access to the brain, leading to a lower response. A direct interaction between morphine and methadone is unlikely because at the time methadone analgesia is measured, the morphine levels are undetectable.

Table 2. Mean AUC_{0-240} from individual data (analgesic effect–time) after intravenous methadone (0·35 mg kg^{-1}, 12 h after morphine withdrawal) in control (n = 10) and rats with abstinence syndrome (n = 13).

	AUC (s min)
Control	401·0 ± 37·06
Abstinence	287·6 ± 24·81*

*$P < 0.05$ compared with control.

In summary our results could explain, at least in part, the interindividual differences observed in methadone response.

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