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Effect of P-glycoprotein inhibition on methadone analgesia and brain distribution in the rat

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Abstract

Methadone is an opiate drug that has been identified as an in-vitro substrate of the efflux pump P-glycoprotein (P-gp), active in the intestinal epithelium and in the blood–brain barrier (BBB), among other sites. The objective of this study was to test in vivo, in the rat model, the role of P-gp modulation on the analgesic effect and brain uptake of methadone, as well as identify the most relevant site via dual oral and intravenous (i.v.) experiments. The P-gp specific inhibitor (valspodar or PSC833) was preadministered (10 mg kg^{-1} i.v.) to test groups. Analgesia was measured using the tail-flick test. The ED_{50} for oral methadone (2, 3, 6 and 8 mg kg^{-1}) decreased three-fold in valspodar groups compared with controls (2.23 ± 0.002 mg kg^{-1} and 6.07 ± 0.07 mg kg^{-1}; $P < 0.0001$). The overall analgesic effect (% antinociception) was elevated 3.1 times in pretreated compared with control rats (90.65% ± 0.22 vs 29.23% ± 14.0; $P < 0.01$) after 6 mg kg^{-1} oral methadone and 2.8 times after i.v. (0.35 mg kg^{-1}) administration (91.75% ± 4.27 vs 32.45% ± 9.0; $P < 0.01$). The brain:plasma distribution ratio was higher in pretreated animals and AUC_{brain} (overall brain concentration) was 6 times higher after oral methadone and 4 times higher after i.v. compared with controls, disproportionally increased relative to plasma, implying an active process at the BBB. P-gp, and hence substrate comedication, plays a critical role in the evolution of the methadone analgesic effect and in its brain uptake, independent of the administration route.

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Introduction

P-glycoprotein (P-gp), an ATP-binding cassette member, acts as a transmembrane efflux pump with broad specificity for structurally diverse xenobiotics. It is particularly active with hydrophobic drugs, which are common substrates for this protein. The importance of P-gp was first recognized in cancer cells, where it is responsible for the development of resistance to cytotoxic agents. It is also expressed in normal tissues and is abundant in epithelial cells with a barrier function such as the enterocytes of the small intestine, in lymphocytes and in the capillaries of the blood–brain barrier (BBB) (Tanigawara 2000).

In-vitro studies using caco-2 cells (where P-gp is highly expressed) have demonstrated that it plays a significant role in interacting with the absorption of several drugs (Augustijns et al 1993; Burton et al 1993; Hunter et al 1993). The direct influence of P-gp on bioavailability has also been evaluated in vivo by the administration of substrates to multiple drug resistance [*mdr1a (−/−)*] knockout mice (Sparreboom et al 1997). This gene expresses P-gp. Studies in humans are more complex and data are often derived indirectly from inhibition studies (Meerum Terwgot et al 1999; Malingre et al 2001), i.e. administration of substrates together with known inhibitors of P-gp (Verschraagen et al 1999).

P-gp directly relates to the bioavailability of drugs and its action modulates their tissue distribution and pharmacokinetics (PK) (Schinkel et al 1996). Also, because of its presence in the BBB, a reduction of functional protein there could lead to highly increased brain distribution of P-gp substrates (Taylor 2002). In a study involving healthy volunteers receiving loperamide (a potent opiate drug that does not produce central effects) and loperamide together with quinidine (a known relatively selective P-gp inhibitor), the opiate drug produced no respiratory response when administered

alone, but respiratory depression occurred when it was given with quinidine (Sadeque et al 2000). This was not explained by the plasma concentration (Cp) levels of loperamide but a lack of relationship between Cp and effect was found, suggesting a role for P-gp in the BBB. In fact, it has been shown that loperamide and other naturally occurring, as well as synthetic, opiate analgesics may belong to a general class of P-gp substrates (Callaghan & Riordan 1993).

Methadone is a synthetic opiate, used for several years in opioid substitution therapy and in the treatment of chronic pain, with known inter-patient variability in the response, mainly after oral dosing. Despite the long time use of methadone in the clinic, interest still exists in identifying the sources of variability in the response to methadone. These could be related to the pharmacodynamics (PD) (i.e. genetic variability in the mu-receptor gene), to the PK (e.g. variability of the CYP450 metabolizing enzyme family), to protein binding, or to the activity of P-gp (Eap et al 2002; MacPhee et al 2002).

In in-vitro models, methadone was shown to be a substrate of P-gp in the intestine (Bouër et al 1999), suggesting a possible role of this efflux protein in methadone's interindividual variability. Although in a recent study with *mdr1a/b* (−/−) knockout mice an increase in methadone analgesia (hot plate) was observed (Thompson et al 2000), until now there has been no evidence of the contribution of in-vivo P-gp modulation on methadone's disposition and effect. The present study examines the possible changes after the in-vivo acute inhibition (by valspodar), in the response of rats to oral as well as intravenous (i.v.) methadone, using the analgesic effect as an endpoint. The role of P-gp in the brain distribution of oral and i.v. methadone is also examined in-vivo.

Valspodar (or PSC833), a ciclosporin derivative, is highly effective in selectively reversing P-gp-mediated multiple drug resistance (MDR) activity, and also in reversing the physiological function of P-gp in various tissues, including the brain, liver, kidney and intestine. Studies to elucidate the mechanism of action of PSC-833 indicated that its potency and specificity might be due to its high affinity for P-gp and the inhibition of P-gp ATPase activity (Atadja et al 1998; Kawahara et al 2000; Martinez & Amidon 2002).

Materials and Methods

Chemicals

Valspodar (PSC833) was kindly supplied by Novartis Pharma (Basel, Switzerland) and was dissolved in a vehicle containing 5% Cremophor EL (a derivative of castor oil and ethylene oxide; Sigma Aldrich, Steinheim, Germany), 1% ethanol and 94% saline. Methadone (racemic mixture) chlorhydrate was supplied by Alcaciber (Madrid, Spain) and ^{14}C methadone was purchased from Amersham Life Science (Buckinghamshire, UK). Biosol (Biodegradable Tissue Solubilizer) and Bioscint (Liquid Scintillation Solution) were purchased from National Diagnostics (Atlanta, GA).

Animals

Sprague–Dawley male rats were used, weighing 180–225 g, as supplied by the University of the Basque Country. Animals were kept under a controlled temperature of 20 °C and humidity of 70% with a normal 12-h light/dark cycle. All experiments were started between 8:30 and 10:30 a.m. to exclude the influence of circadian rhythms. The experimental protocol was approved by the Committee on Animal Experimentation of the University of the Basque Country. The day before the experiment, rats were lightly anaesthetised with ether, and a polyethylene catheter (i.d. 0.3 mm, 10 cm length; Vygon, France) was implanted in the right jugular vein for the i.v. administration of the drugs. All catheters were filled with a solution containing NaCl 0.9% (Panreac, Barcelona, Spain) and 1% heparin (50 IU mL^{-1}, Chiesi Wassermann, Barcelona, Spain). The catheters were tunnelled under the skin of the animal and externalised on the dorsal surface of the neck. After the surgery, all rats were kept under fasting conditions but with ad libitum access to water.

Experiment I: analgesic assay

Analgesia was measured by the tail-flick method (Le Bars et al 2001) after both oral and i.v. administration of methadone. The rats responded to a heat stimulus by moving their tail away from the focus of the stimulus, thereby exposing a photocell located under the tail, and reaction time was automatically recorded. The intensity of the heat was adjusted so that the basal measurements were of 2–5 s; rats with baseline latencies longer than 5 s were excluded. Baseline tail-flick latencies were recorded at 10 min before and immediately before opiate administration. A maximal cut-off time of 10 s was used to prevent tissue damage. The time evolution of the analgesic effect was measured for 3 h after oral administration of methadone and for 2 h after i.v. Animals were randomly distributed in two groups, valspodar and control groups.

Valspodar group

Valspodar (10 mg kg^{-1}) was infused i.v. to rats over 5 min, 30 min before administration of oral methadone, at doses of 2 mg kg^{-1} (n = 6), 3 mg kg^{-1} (n = 4), 6 mg kg^{-1} (n = 4) and 8 mg kg^{-1} (n = 6) and i.v. at 0.35 mg kg^{-1} (n = 4). This i.v. methadone dose was found to elicit analgesia with minor adverse respiratory effects (Garrido et al 1999).

The suspension vehicle for valspodar (vehicle group) was also tested for possible analgesia in the absence of the active ingredient; it was infused (over 5 min) i.v. to rats 30 min before the administration of methadone through the oral route at doses of 2 mg kg^{-1} (n = 3), 3 mg kg^{-1} (n = 3), 6 mg kg^{-1} (n = 3) and 8 mg kg^{-1} (n = 3) and through the i.v. route at 0.35 mg kg^{-1} (n = 5).

Control group

Animals in this group received methadone at doses of 2 mg kg^{-1} (n = 4), 3 mg kg^{-1} (n = 4), 6 mg kg^{-1} (n = 6) and 8 mg kg^{-1} (n = 5) through the oral route, and 0.35 mg kg^{-1} (n = 8) i.v.

Experiment II: disposition of methadone in the brain and protein binding

As in Experiment I, animals were randomly distributed in to the two groups.

Valspodar group

Valspodar (10 mg kg^{-1}) was infused i.v. to rats over 5 min, 30 min before administration of radiolabelled methadone (^{14}C methadone) 6 mg kg^{-1} via oral route (n = 18) or i.v. at 0.35 mg kg^{-1} (n = 22).

Control group

Animals in this group received radiolabelled methadone (^{14}C methadone) orally 6 mg kg^{-1} (n = 19) or i.v. at 0.35 mg kg^{-1} (n = 19).

These methadone doses correspond to the ED_{50} for the oral route (data from Experiment I) and to the analgesic dose for the i.v. route (data from previous experiments).

The animals were then sacrificed by decapitation under light ether anaesthesia at 15, 20, 30, 45, 90 and 240 min after oral drug administration, and at 1, 2, 3, 5, 20 and 45 min after i.v. administration; three to four animals were used at each time point. In order to calculate the brain:plasma distribution ratios, trunk blood was also collected at the same time points.

Methadone brain and blood levels were measured in both groups using an already described experimental protocol (Garrido et al 1999) with minor modifications. The brains were rapidly removed and 50 mg of brain (cortex) was dissected. Tissue samples (50 mg) were mixed with 1 mL of Biosol Tissue Solubilizer, and were incubated for 1.5 h (time needed for the complete tissue digestion) at 50 °C; 10 mL of Bioscint Liquid Scintillation Solution was added when the solution was at room temperature. Radiolabelled methadone concentrations were measured by scintillation counting using a Packard model tri-Carb Liquid Scintillation Analyser.

Blood samples were centrifuged during 15 min (2500 rpm, 37 °C); 50 μL of plasma was placed in plastic scintillation vials and 10 mL of Bioscint Liquid Scintillation Solution was added for posterior quantification.

^{14}C methadone plasma protein binding was determined at 37 °C by ultrafiltration using a micropartition system (Amicon MPS-1 device). An aqueous solution (10 μL) of ^{14}C methadone was added in vitro to 990 μL of plasma, from control (n = 3) and valspodar (10 mg kg^{-1}) pre-treated rats (n = 3), to a final methadone concentration of 70 ng mL^{-1}; 50 μL of this final solution was collected in order to calculate methadone total concentration (C_T). The 950 μL left was incorporated into the devices, and centrifuged at 2500 rpm for 15 min at 37 °C. A volume of 50 μL of the ultrafiltrate was collected to measure the unbound methadone concentration (C_u) by scintillation spectrometry. The percentage of unbound methadone (fu) was determined as:

$$\%\ \text{unbound} = \frac{C_u}{C_T} \times 100 \qquad (1)$$

This procedure was developed according to the validated method described by Gómez et al (1995).

Data analysis

The degree of analgesia was calculated by two methods:

(a) Using the area under the curve of tail-flick latency against time (s), which yields an overall value of the antinociception induced over the experimental period relative to the maximum reachable, according to the formula:

$$\%\ \text{antinociception} = \left(\frac{AUCE_d - AUCE_b}{AUCE_{max} - AUCE_b}\right) \times 100 \quad (2)$$

where $AUCE_d$ and $AUCE_b$ are the areas under the effect–time curve (calculated from 0–180 or 0–120 min according to the administration route) for drug-treated and basal response animals, respectively; $AUCE_{max}$ is the area under the curve of maximum possible antinociception (10 s in each determination). The calculation of the AUCE of tail-flick latency against time was carried out using the trapezoidal method aided by Graph Pad (version 3.0, Graph Pad Software Inc., San Diego, CA).

(b) Directly using latency time values according to the formula:

$$MPR\% = \left(\frac{\text{test latency} - \text{baseline latency}}{\text{cutoff time} - \text{baseline latency}}\right) \times 100 \quad (3)$$

where test latency and baseline latency are the tail-flick latency times after methadone and basal latency time of each animal (before methadone administration), respectively, and cut-off time is the maximum latency time (10 s).

The ED_{50} (dose of methadone that produces half of the maximum effect) values were calculated from the dose–response (% antinociception, equation 2) curves using non-linear regression analysis with WinNonLin (Pharsight Inc., NC).

The brain:plasma distribution ratio was calculated at all time points dividing methadone brain levels by their corresponding plasma concentrations. The area under the brain concentration vs time curve (AUC_{brain}) was also calculated using the trapezoidal method.

Two-tailed unpaired Student's *t*-test and ANOVA with Bonferroni post-hoc tests were used to check for significant differences between groups. Values of $P < 0.05$ were considered statistically significant.

Results

The dose–response curve for oral methadone administration (2, 3, 6 and 8 mg kg^{-1}), expressed as a percentage of antinociception (equation 2), is shown in Figure 1 for the four doses of methadone given to rats with and without concomitant administration of i.v. valspodar (10 mg kg^{-1}). The figure shows the mean observed values for each methadone

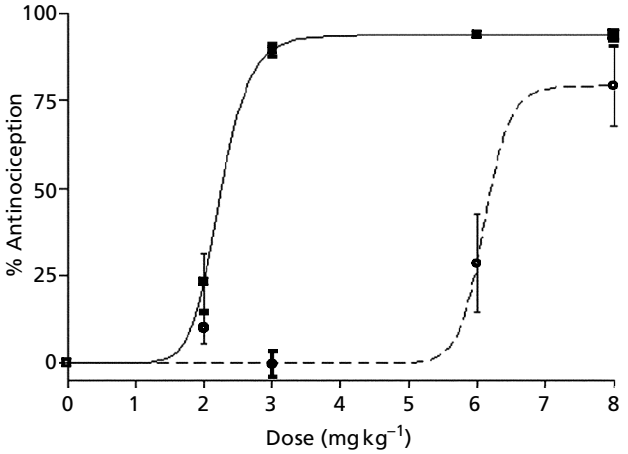

Figure 1 Dose–response curve (best model fit) for oral methadone alone (2, 3, 6 and 8 mg kg^{-1}) (n = 19; ---O---) and identical methadone doses plus 10 mg kg^{-1} valspodar (n = 20; —■—). Each point represents the mean (percentage of antinociception as calculated from equation 2) ± s.e.m.

dose and the best model fit. Pretreatment with valspodar produced a significant decrease in methadone's ED_{50} (6.07 ± 0.07 mg kg^{-1} for the control group vs 2.23 ± 0.002 mg kg^{-1} for the valspodar group; $P < 0.0001$). The presence of valspodar's vehicle produced no significant change in ED_{50} (6.07 ± 0.07 mg kg^{-1} and 6.11 ± 0.09 mg kg^{-1} in the control and vehicle pretreated animals respectively). Subsequently, the 6 mg kg^{-1} (similar to ED_{50}) oral dose of methadone was used to carry out the rest of the experiments.

Figure 2A shows the observed temporal evolution (0–180 min) of the analgesic effect (%MPR as calculated from equation 3) of oral methadone (6 mg kg^{-1}) in rats with concomitant i.v. administration of valspodar, and also controls without valspodar. Rats receiving the P-gp inhibitor displayed significantly higher levels of analgesia at all time points except for 5, 10 and 30 min, compared to the controls who took only methadone.

Figure 2B shows that, at this methadone dose, the overall antinociception (expressed as the percentage of antinociception; equation 2) was significantly higher in valspodar-pretreated animals (29.23 ± 14.0% vs 90.65 ± 0.22% of antinociception in control and pretreated animals respectively; $P < 0.01$). In this particular case, the overall antinociception was calculated from 0–120 min in order to compare with i.v. administration data. No differences were found between control and valspodar-vehicle pretreated animals (29.23% vs 21.4% antinociception respectively; $P > 0.05$).

The brain concentration vs time data (expressed as μg of ^{14}C methadone per gram of brain tissue) for both control and valspodar-pretreated rats after a 6 mg kg^{-1} oral dose of methadone are shown in Figure 3A. Methadone brain levels were higher in the valspodar group after oral administration of the opiate, being statistically significant at 30, 90 and 240 min ($P < 0.05$). The

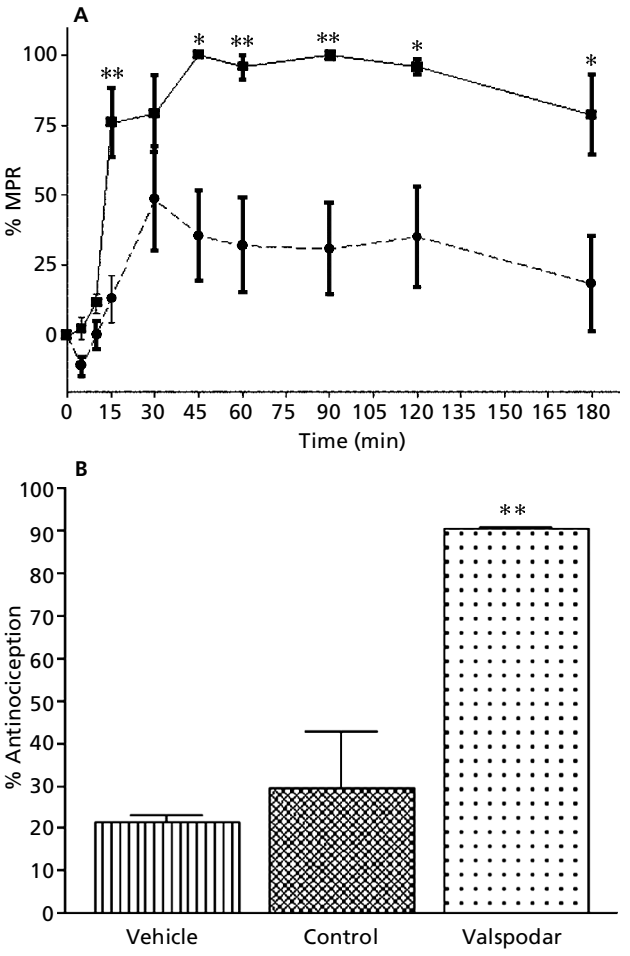

Figure 2 (A) Observed time course of analgesic effect (%MPR, see Materials and Methods, equation 3) after oral methadone (6 mg kg^{-1}) in control rats (n = 6 for all time points; ---O---) and the same dose of methadone with concomitant i.v. administration of valspodar (10 mg kg^{-1}) over 5 min (n = 4 for all time points; —■—). Valspodar-pretreated animals showed significantly higher analgesia at indicated time points (*$P < 0.05$, **$P < 0.01$). Bars are standard errors of the mean (s.e.m.). (B) Effects of 6 mg kg^{-1} oral methadone in valspodar vehicle (▥), control (▩) or valspodar-pretreated (▨) rats. Each column represents the mean ± s.e.m. (n = 3, 6, 4, respectively). The percentage of antinociception was calculated according to equation 2, in this particular case AUCE was calculated from 0–120 min. Significant differences were found when comparing the effect in control animals with valspodar-pretreated rats (**$P < 0.01$). No differences were found between control and valspodar vehicle animals ($P > 0.05$).

overall brain concentration, expressed as $AUC_{brain0-240}$, showed a six-fold increase in the pretreated group when compared with the control group (2685 vs 472.8 μg/g min respectively). The data related to the in-vivo determination of brain:plasma distribution ratios are shown in Figure 3B. Pretreatment with valspodar produced a significantly higher brain uptake of methadone at all times except for at 20 min.

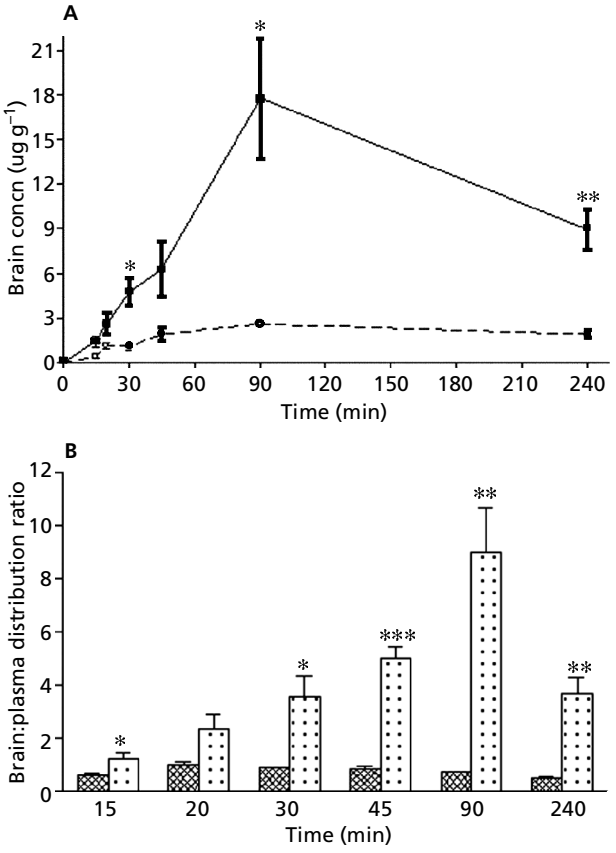

Figure 3 (A) Brain tissue levels of ^{14}C methadone (6 mg kg^{-1}) in control rats (n = 19; ---O---) and valspodar-pretreated (10 mg kg^{-1} i.v.) rats (n = 18; —■—). Each point represents the mean ± s.e.m., data are expressed as μg of ^{14}C methadone per gram of brain tissue. Statistically significant differences between groups were found at 30, 90 and 240 min after methadone administration (*$P < 0.05$, **$P < 0.01$). (B) In-vivo determination of brain:plasma distribution ratios at different times following oral methadone administration (6 mg kg^{-1}) in control (▩) and valspodar-pretreated (▨) animals. Data are presented as the mean ± s.e.m. (n = 3–4 for each point; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

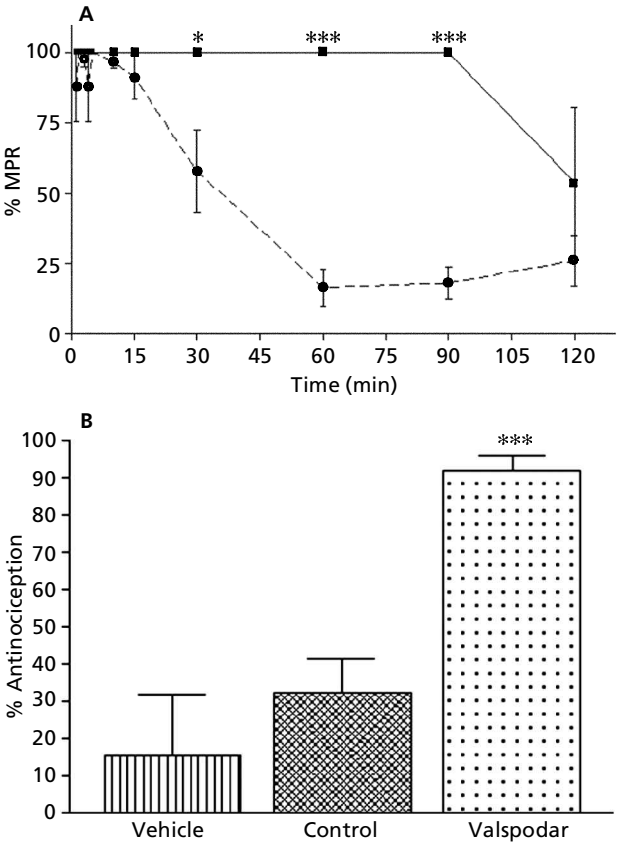

Figure 4 (A) Observed time course of analgesic effect (%MPR, see Materials and Methods, equation 3) in control rats (n = 8 for all time points; ---O---) receiving 0.35 mg kg^{-1} of methadone i.v. and in rats pretreated with valspodar (10 mg kg^{-1} i.v.) (n = 4 for all time points; —■—) 30 min before the same i.v. dose of methadone. Valspodar-preteated animals showed significantly higher analgesia at indicated time points (*$P < 0.05$, ***$P < 0.001$). Bars are standard errors of the mean. (B) Antinociceptive effects of 0.35 mg kg^{-1} i.v. methadone in valspodar vehicle (▥), control (▩) or valspodar-pretreated rats (▨) (n = 5, 8, 4, respectively). The percentage of antinociception was calculated using equation 2 (see Materials and Methods). Each column represents the mean ± s.e.m. Significant differences were found when comparing antinociception in control rats with valspodar-pretreated animals (***$P < 0.001$). No differences were found between control and valspodar vehicle groups ($P > 0.05$).

Figure 4A shows the observed temporal evolution of the analgesic effect (%MPR calculated according to equation 3) of i.v. methadone (0.35 mg kg^{-1}) after concomitant administration of i.v. valspodar, and also controls without valspodar. Pretreated rats show higher analgesia levels at all measurement times. Statistical differences were found at 30, 60 and 90 min. In Figure 4B the total effect is shown as percentage antinociception (equation 2). This percentage was significantly elevated in the valspodar-treated group compared with the control (91.75 ± 4.27% vs 32.45 ± 9.0%, respectively; $P < 0.01$). There were no differences between the control and valspodar vehicle groups (19% vs 15% respectively; $P > 0.05$).

The brain concentration vs time data for both control and valspodar-pretreated rats after a 0.35 mg kg^{-1} i.v. dose of methadone are shown in Figure 5A. Methadone brain levels were higher at all time points, showing a significant increase in the valspodar group at 20 and 45 min after i.v. administration of the opiate. The overall $AUC_{brain0-45}$ was 86.08 vs 21.88, a four-fold increase compared with the control group. The brain:plasma distribution ratio for methadone is shown in Figure 5B where a significant increase is seen in the uptake at 20 min.

In order to determine if changes in the drug effect could be partially explained by alterations in methadone's protein binding caused by valspodar pretreatment, methadone's unbound fraction was calculated for both groups of rats. As shown in Figure 6, valspodar produced no change in methadone's binding to plasma proteins.

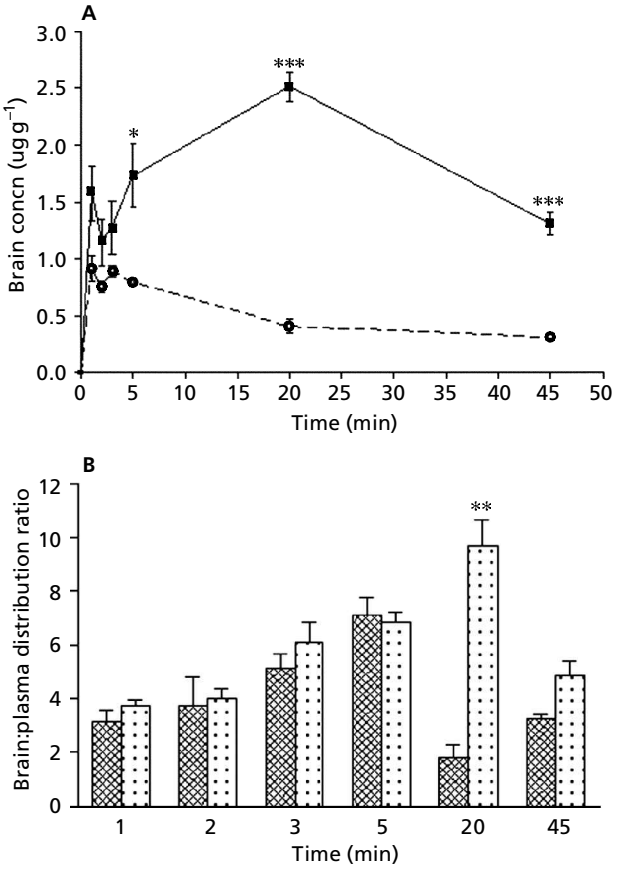

Figure 5 (A) Brain tissue levels of ^{14}C methadone (0.35 mg kg^{-1}; i.v.) in control rats (n = 19; ---○---) and valspodar-pretreated (10 mg kg^{-1}) rats (n = 22; —■—). Points are mean ± s.e.m., data are expressed as μg of ^{14}C methadone per gram of brain tissue. Significant differences between groups were found at 5, 20 and 45 min after methadone administration (*$P < 0.05$, ***$P < 0.001$). (B) In-vivo determination of brain:plasma distribution ratios at different times following methadone i.v. administration (0.35 mg kg^{-1}) in control (▩) and valspodar-pretreated (▨) animals. Data are presented as the mean ± s.e.m. (n = 3–4 for each time point) (**$P < 0.01$).

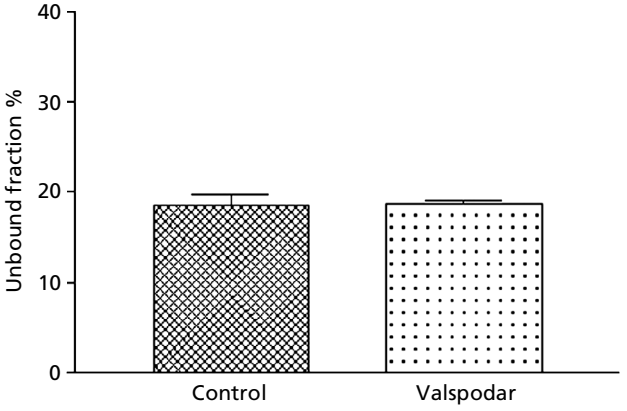

Figure 6 Methadone unbound fraction was measured with the ultrafiltration technique both in control (▩) and valspodar-pretreated (▨) rats. Data are mean ± s.e.m. (n = 3 rats per group). No significant differences were found in methadone protein binding between groups ($P > 0.05$).

Discussion

Methadone displays large interindividual variability in its PK and apparently also in its PD, with several contributing factors already identified yet not always quantified (Eap et al 2002). In previous work, the focus was on variables such as sex (Rodriguez et al 2002), plasma protein binding (Garrido et al 1999) and concomitant drug administration (Carlos et al 2002) but these factors do not entirely explain the large interindividual variability in the effect of methadone. In-vitro studies have suggested that methadone may be a substrate of intestinal P-gp (Bouër et al 1999) and therefore modulation at this level could contribute to the wide variation observed in methadone bioavailability ranging from 36 to 100% (Nilsson et al 1982; Gourlay et al 1986). However, depending on the experimental model used, results from different studies can have distinct interpretations, possibly due to the overlooked influence of epithelium proteins and enzymes in absorption.

Here, in the in-vivo rat model, the influence of P-gp in the modulation of methadone analgesia was tested and brain uptake was quantified using a specific (with respect to CYP3A4 but possibly not other ATP members) P-gp inhibitor (Bourasset et al 2003). All analyses were made after oral and intravenous methadone. Comparison of the oral and i.v. routes reveals the level at which the interaction is active.

Oral methadone potency was increased three-fold in the valspodar-pretreated group. This increase in the analgesic effect of methadone in P-gp-inhibited animals may be due to P-gp modulation at the intestinal site and/or at other levels, mainly BBB. Specifically, after 6 mg kg^{-1} oral methadone the overall effect, expressed as the percentage of antinociception, was 90.65 ± 0.22% in rats pretreated with valspodar and 29.23 ± 13.64% in controls. Moreover, when we used an equipotent intravenous dose of methadone (0.35 mg kg^{-1}) it was 91.75 ± 4.27% in pretreated vs 32.45 ± 9.0% in control animals. These results suggest a critical role of P-gp in methadone's analgesic effect after both oral and intravenous methadone administration, and seem to indicate that the interaction between P-gp and methadone takes place at some level beyond the intestinal, such as the BBB. Remarkably, the increase in the analgesic effect, after selective P-gp inhibition, was 3.1 and 2.8 times after oral and i.v. methadone administration respectively. It would appear then, as some authors have already suggested, that: (a) methadone could be a weak substrate of intestinal P-gp and (b) the brain could be more sensitive to changes in the function of P-gp than other tissues (Lin & Yamazaki 2003).

In order to establish the role of P-gp in methadone's brain disposition a second experiment was carried out

consisting of the i.v. (0.35 mg kg^{-1}) and oral administration (6 mg kg^{-1}) of ^{14}C methadone in control and P-gp-inhibited animals. ^{14}C methadone was used since the metabolites of this drug are inactive and do not reach the brain (Liu et al 1983). Moreover, in previous work it has been shown that brain levels of ^{14}C methadone, in control rats, are related to the analgesic effect (Garrido et al 1999).

The brain levels of methadone were superior, over all time points, in rats pretreated with valspodar independent of administration route, explaining the observed change in the analgesic effect. Interestingly, the integrated brain methadone concentration (AUC_{brain}) was increased in pretreated animals 6 times after oral and 4 times after i.v. administration, indicating a critical role for P-gp in BBB. However, the magnitude of this increase did not parallel that observed in the analgesic effect (three-fold increase in pretreated animals in both administration routes). The higher brain concentrations needed after oral administration to reach the same analgesic effect may be explained by diverse mechanisms, e.g. different brain efflux/influx rate between oral and i.v. administration, which could contribute to an irregular distributional process across the brain tissue, and thus favour the binding of the drug to non-specific structures. Future investigations are needed to elucidate the mechanism involved.

In the present study, modulation of the methadone brain:plasma distribution ratio was finally confirmed by quantifying methadone levels in brain tissue and plasma collected at different times after dosing and by calculating the ratio at all times. This brain:plasma distribution ratio serves as a measure of altered tissue selectivity (BBB) since this value is normalized by the respective blood level. Indeed, the ratio was higher in pretreated rats independent of administration route. Methadone's brain uptake reached approximately the same value in the presence of valspodar, in minute 20 after i.v. administration of methadone, and 90 min after its oral intake, times at which equilibrium had been reached in both cases. The fact that this ratio is greater than unity in pretreated groups confirms that an active rather than a passive process is operative at the BBB.

The possibility that part of the increase in brain uptake of methadone following valspodar treatment could be due to displacement of protein-bound methadone was rejected. The methadone unbound fraction was not significantly different between groups ($18.54 \pm 1.15\%$ vs $18.83 \pm 0.21\%$ in control and valspodar-pretreated animals, respectively).

The work presented here indicates that P-gp plays an important role in the distribution of methadone to the central nervous system. Similar results have already been observed for other drugs such as anticonvulsants (Cox et al 2002), HIV protease inhibitors (Huisman et al 2001) and opiate drugs (Sadeque et al 2000), including morphine (Letrent et al 1999), but no information previously existed for methadone.

Conclusion

A critical role was observed for P-gp in the evolution of the methadone analgesic effect and in its brain uptake, pointing to the potential for adverse drug–drug interactions and the need to include P-gp activity and possibly interacting comedication as a covariable in future PK and PD studies.

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