Using \([^{11}\text{C}]\text{Diprenorphine}\) to Image Opioid Receptor Occupancy by Methadone in Opioid Addiction: Clinical and Preclinical Studies

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ABSTRACT

Substitute methadone prescribing is one of the main modes of treatment for opioid dependence with established evidence for improved health and social outcomes. However, the pharmacology underpinning the effects of methadone is little studied despite controversies about dosing in relation to outcome. We therefore examined the relationship between methadone dose and occupation of opioid receptors in brain using the positron emission tomography (PET) radioligand \([^{11}\text{C}]\text{diprenorphine}\) in humans and rats. Eight opioid-dependent subjects stable on their substitute methadone (18–90 mg daily) had an \([^{11}\text{C}]\text{diprenorphine}\) PET scan at predicted peak plasma levels of methadone. These were compared with eight healthy controls. No difference in \([^{11}\text{C}]\text{diprenorphine}\) binding was found between the groups, with no relationship between methadone dose and occupancy. Adult male Sprague-Dawley rats that had been given an acute i.v. injection of methadone hydrochloride (0.35, 0.5, 0.7, or 1.0 mg kg\(^{-1}\)) before \([^{11}\text{C}]\text{diprenorphine}\) showed a dose-dependent increase in biodistribution but no reduction in \([^{11}\text{C}]\text{diprenorphine}\) binding. We suggest that the lack of a dose-dependent relationship between methadone dose, either given chronically in human or acutely in rat, and occupancy of opioid receptor measured with \([^{11}\text{C}]\text{diprenorphine}\) PET is related to efficacy of this opioid agonist at very low levels of opioid receptor occupancy. This has implications for understanding the actions of methadone in comparison with other opioid drugs such as partial agonists and antagonists.

Opiate addiction is a chronically relapsing illness and a major health problem, with large direct health costs, both psychiatric and physical, as well as significant costs to society in terms of crime, loss of earnings and productivity, and social damage (Mark et al., 2001). One of the few proven treatments is the use of substitute or maintenance therapy with the opioid agonist methadone, which has been used successfully for several decades in the United States and the United Kingdom. There is substantial evidence to support the effectiveness of methadone in reducing illicit opioid use, intravenous drug use, crime, unemployment, and improving overall health status, social rehabilitation, and quality of life (Marsch, 1998). However, methadone treatment is not without its problems. Apart from the perceived stigma of being addicted to an opioid, many patients still relapse and fundamental questions about the mechanisms of action of methadone remain unanswered. For example, it is not well understood why some patients seem to require larger doses to suppress the desire to use or to help them move away from use of heroin (Maxwell and Shinderman, 2002). Interindividual differences in the pharmacokinetics and pharmacodynamics of methadone globally (Eap et al., 2002) and at the blood-brain barrier (Wang et al., 2004) may only partially explain these differences.

Despite the extensive use of the methadone over 20 years, little is known about the relationship between dose, brain levels, and receptor occupation. A long-held underlying assumption is that substitution medication, such as methadone, occupies opioid receptors in the brain and thus minimizes withdrawal symptoms and prevents ’on-top’ heroin use from accessing the receptor, thus reducing its reinforce-
ment. We have previously shown that methadone-maintained opioid-dependent patients are less sensitive to the objective and subjective effects of hydromorphone, an opioid agonist (Melichar et al., 2003a). Information about the dose: opioid receptor occupancy relationship for methadone should help address important clinical issues such as the correlation of dose to outcome and the assessment of patients who claim they are being underdosed.

[11C]Diprenorphine has been used extensively as a PET neuroimaging ligand. It has high affinity at µ, κ, and δ opioid receptors (Jones et al., 1988; Sadzot et al., 1991), being an antagonist at µ and δ but having weak efficacy at the κ opioid receptor, where it acts as a partial agonist (Lewis and Husband, 2004). It was originally chosen as a positron emitting radioligand because of its safety, lack of side-effects at tracer doses in human pilot studies, rapid cerebral uptake, and high percentage (80–90%) of specific binding in animal in vivo studies. In rat, it is most densely distributed in the basal ganglia and neocortex, excluding the occipital cortex (Pert et al., 1975). In human, in vivo, high uptake was observed in [11C]diprenorphine has been demonstrated with PET in regions such as the thalamus, caudate nucleus, and temporal, frontal, and parietal cortices, which are known from post-mortem studies to have high concentrations of µ, κ and δ opioid receptors (Jones et al., 1988). The aim of this current study, therefore, was to examine the relationship between methadone dose and occupancy of brain opioid receptor levels, as measured by [11C]diprenorphine PET in humans and rats.

**Materials and Methods**

**Human Study**

**Subjects.** Eight male opioid-dependent patients, stabilized on methadone, were recruited from patients attending the methadone clinic at the Bristol Specialist Drug Service in Bristol, UK. They fulfilled the Diagnostic and Statistical Manual of Mental Disorders-IV criteria for opioid dependence and were only consuming methadone. They were typically long-standing opioid users, had been on methadone for a mean of 5.9 years (range 1–20 years) and had a mean age of 39.9 (range 20–46), and had been on a stable dose for at least 1 month (Table 1). Subjects were not abusing other drugs, as demonstrated by regular urine drug screens, and all smoked nicotine. They had no serious physical or mental illness, as demonstrated by clinical interview and physical assessment. These subjects were compared with eight age-matched control subjects (six males, two females), mean age 37 years (range 25–56), who had no significant mental or physical illness and were not abusing any drugs, as demonstrated by a urine drug screen (two were nicotine smokers).

**Methods.** The opioid-dependent patients received under supervision their usual methadone dose 4 h before their [11C]diprenorphine PET scan. This was to provide a measure of peak methadone occupancy at the opioid receptors. [11C]Diprenorphine was synthesized according to standard protocols (Luthra et al., 1985). Scans were obtained using a brain-dedicated ECAT 953b Siemens/CTI PET scanner in high-sensitivity three-dimensional mode. The dose of [11C]diprenorphine per scan was of the order of 370 MBq given as an intravenous bolus (Jones et al., 1994). An arterial line was inserted into the nondominant radial artery to allow [11C]diprenorphine metabolites to be measured every 5 to 10 min to generate a radiolabeled metabolite-corrected input function. The images were acquired over 90 min as 18 time frames and were reconstructed into 31 transaxial planes using filtered back projection, which were then analyzed.

For six of the methadone group, blood samples were analyzed for their (R)-enantiomeric (active compound) and (S)-enantiomeric methadone levels. Blood samples were taken 4 h after supervised ingestion of their usual daily dose of methadone, to provide a measure of peak methadone levels, just before the injection of [11C]diprenorphine. The blood samples were analyzed by high-performance liquid chromatography with a chiral column as described previously (Eap et al., 1996).

This study was approved by the local Research Ethics Committees and by the UK Administration of Radioactive Substances Advisory Committee. After a full explanation of the protocol, all volunteers gave written informed consent.

**Animal Study**

The work was carried out by licensed investigators, according to the Home Office’s Guidance in the Operation of Animals (Scientific Procedures) Act 1986 and used 34 adult male Sprague-Dawley rats with a body weight of 292 ± 3 g (mean ± S.E.). During the study, the rats were awake but lightly restrained in Bollman cages.

**Methods.** Each rat was given −11 MBq of [11C]diprenorphine in 0.20 ml, via a previously catheterized tail vein. At designated times after injection, rats were given an i.v. injection of Euthatal, the brain was removed, and tissues were rapidly sampled into preweighed vials. The tissues were cervical cord, olfactory tubercles, hypothalamus, thalamus, prefrontal cortex, striatum, somatosensory cortex, hippocampus, visual with temporal cortex, inferior colliculi, superior colliculi, and medulla with pons and cerebellum.

In the first series of experiments (“time:course”), sample times were 1, 5, 20, 30, 45, 60, and 90 min after injection for a control group; and for a group of rats given racemic methadone hydrochloride (0.35 mg kg⁻¹ i.v.; Sigma-Aldrich, St. Louis, MO), 5 min before [11C]diprenorphine. A single animal was used per time point. In six of the rats, blood was collected at graded times via a previously catheterized tail artery (three control and three predosed with methadone, six samples per rat). The 0.35 mg kg⁻¹ i.v. dose was chosen as one which elicits “analgesia with minimum adverse respiratory effects” (Garrido et al., 1999). Using this dose, the authors quote a rat plasma concentration of −100 ng ml⁻¹ immediately after injection, falling rapidly to −50 ng ml⁻¹ within 5 min, and then more gradually to −20 ng ml⁻¹ at 60 min. The corresponding brain (cortex) concentration was −350 ng g⁻¹ at 5 min after injection, falling to −150 ng ml⁻¹ at 20 min.

**TABLE 1**

Demographic information for the eight male methadone addicts

<table>
<thead>
<tr>
<th>Wt (kg)</th>
<th>Age (yr)</th>
<th>Methadone Dose (mg)</th>
<th>Duration of Opioid Addiction (yr)</th>
<th>Duration of Current Methadone Treatment (yr)</th>
<th>Time on Current Methadone Dose (units/week)</th>
<th>Alcohol Intake (grams per week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>20</td>
<td>18</td>
<td>3</td>
<td>1</td>
<td>0.08</td>
<td>20</td>
</tr>
<tr>
<td>77</td>
<td>46</td>
<td>30</td>
<td>25</td>
<td>8</td>
<td>8</td>
<td>28</td>
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<tr>
<td>71</td>
<td>41</td>
<td>50</td>
<td>23</td>
<td>6</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>85</td>
<td>44</td>
<td>50</td>
<td>25</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>89</td>
<td>42</td>
<td>50</td>
<td>22</td>
<td>3</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>65</td>
<td>37</td>
<td>70</td>
<td>16</td>
<td>2</td>
<td>0.25</td>
<td>4</td>
</tr>
<tr>
<td>78.5</td>
<td>43</td>
<td>90</td>
<td>27</td>
<td>3</td>
<td>0.17</td>
<td>20</td>
</tr>
<tr>
<td>68</td>
<td>46</td>
<td>90</td>
<td>24</td>
<td>20</td>
<td>7</td>
<td>30</td>
</tr>
</tbody>
</table>
g \(^{-1}\) at 60 min after injection. Their estimate for drug potency for analgesia (tail-flick test) was 24 ng ml\(^{-1}\) of plasma, uncorrected for plasma protein binding. In the present study, the predosed rats were pale and nonresponsive, with depressed respiration; recovery began at \(\sim 40\) min.

In the second series ("dose/effect"), the sample time was 60 min after \([^{11}C]\)diprenorphine injection for a control group (\(n = 5\)) and for four further groups of rats given methadone as before, at either 0.35 (\(n = 5\)), 0.5 (\(n = 3\)), 0.7 (\(n = 4\)), or 1.0 mg kg\(^{-1}\) (\(n = 3\)). For presentation, data from control and 0.35 mg kg\(^{-1}\) dosed rats have been added to the first series, giving \(n = 6\), for the 60-min time point.

### Data Analysis and Statistics

**Human Study.** The dynamic images were reconstructed with filtered back projection, measured attenuation, and dual energy window scatter corrections. Each reconstructed frame of \([^{11}C]\)diprenorphine uptake acquired was analyzed on a Sun SPARC workstation (Sun Microsystems, Mountain View, CA) using AnalyzeAVW version 3.1 (Mayo Foundation, Rochester, MN; Robb and Hanson, 1991). Volume of distribution \((V_D)\) images of \([^{11}C]\)diprenorphine were generated with spectral analysis using "receptor parametric mapping" software developed at the MRC Cyclotron Unit, which does not require any reference regions to be used (Gunn et al., 1997). Volumes of interest were drawn on individual \(V_D\) maps of the opioid receptors as labeled by \([^{11}C]\)diprenorphine with reference to the stereotaxic atlas of Talairach and Tournoux (1988). The regions studied included the thalamus, caudate nucleus, and temporal and frontal cortices, known to have high concentrations of opioid receptors. Mean \(V_D\) values were calculated for each volume of interest. These values were then compared between groups using two-tailed \(t\) tests. The relationship between methadone dose, oral daily dose, and \((R)\)-enantiomer methadone level, and availability of opioid receptors was explored.

**Animal Study.** Radioactivity in tissue and blood (whole blood and plasma) was counted using a WALLAC gamma counter with automatic correction for radioactivity decay. Data were normalized for injected radioactivity and body weight, giving "uptake units" \(= (cpm\ g\ \text{g}^{-1}\ \text{wet weight tissue})\ \times (\text{cpm}\ g\ \text{g}^{-1}\ \text{body weight})^{-1}\). The methodology has been described previously (Hume et al., 1992). Specific, rather than total, binding was estimated by expressing tissue radioactivity concentration as tissue/cerebellum ratios minus unity, assuming that rat cerebellum represents a tissue with \(\mu\) site density. The opioid-dependent subjects’ \((R)\)-enantiomer methadone levels are shown in Table 2, alongside their mean global \([^{11}C]\)diprenorphine \(V_D\). Comparison of mean global diprenorphine \(V_D\) showed a nonsignificant trend toward the methadone group having 12% lower levels of \([^{11}C]\)diprenorphine binding than the control group \((17.14 \pm 1.06\ \text{versus}\ 19.54 \pm 1.21, p = 0.10\ \text{uncorrected};\ \text{mean} \pm \text{S.E.})\) (Fig. 1). When individual regions were examined, \([^{11}C]\)diprenorphine \(V_D\) was not significantly lower in any region in opioid-dependent patients compared with control subjects, nor were there any hemispheric asymmetries (Table 3; Fig. 1). The data were further analyzed using published data (Pfeiffer et al., 1982, Cross et al., 1987, Delay-Goyet et al., 1987) on the regional variation in the distribution of the \(\mu\), \(\kappa\), and \(\delta\) opioid receptor subtypes assessed in vitro. This did not yield any relationship between receptor subtype distribution and the effect of methadone on \([^{11}C]\)diprenorphine binding (data not shown).

There was no clear relationship between oral doses or plasma levels (nanograms per milliliter) of the \((R)\)-enantiomer of methadone and \([^{11}C]\)diprenorphine binding, both globally and regionally (Fig. 2).

### Animal Study

**Time Course.** Figure 3 illustrates the effect of 0.35 mg kg\(^{-1}\) methadone hydrochloride (i.v. 5 min before \([^{11}C]\)diprenorphine injection) on the distribution of radioactivity in two of the tissues dissected, namely, thalamus and striatum, chosen as high \(\mu\) site density regions. In these tissues, as in all others dissected, the methadone treatment resulted in a higher initial uptake and retention of \([^{11}C]\)diprenorphine. Also shown in Fig. 3 are the equivalent tissue/cerebellum ratios, taken to represent total/nonspecific binding of the radioligand. When expressed in this way, there was no effect of the methadone predosing, implying that the increased radioactivity concentration reflected a difference in bioavailability of the tracer, rather than an increase in the specific component of binding of \([^{11}C]\)diprenorphine. Consistent with increased bioavailability, we measured a higher plasma radioactivity content in the methadone-treated rats, at early times after injection (data not shown).

Figure 4 summarizes the 60-min data for all tissues dissected. Using a two-factor ANOVA with replication, the radioactivity content (Fig. 4a) was significantly increased, by an average of 16%, in the methadone-treated group (closed columns) compared with control (open columns) \((P < 0.001)\). Evaluation of each tissue using individual ANOVA and Bonferroni correction for multiple comparison showed a statistically significant effect in hippocampus and inferior colliculi. Similar analyses for the tissue/cerebellum ratio data (Fig. 4b) showed no significant group effect and no effect of methadone predosing in any tissue.

**Dose/Effect.** As discussed under Materials and Methods, the 0.35 mg kg\(^{-1}\) dose was chosen as being pharmacologically active and was expected to result in an acute plasma level comparable with that expected during maintenance treat-
ment in patients. Since, however, there is a marked species difference in the pharmacokinetics of methadone (t1/2 = 70–90 min in rats compared with 24 h in human; Zhou et al., 1996) and the rat studies used a racemic mixture, an additional series was included, increasing the methadone up to 1 mg kg$^{-1}$. Again, the methadone was given i.v., 5 min before $[^{11}C]$diprenorphine, but all rats were euthanized at 60 min after radioligand injection. For the same tissues as shown in Fig. 3, Fig. 5 illustrates a graded increase in radioactivity content, as the methadone dose was increased, but again no reduction in the tissue/cerebellum ratios. Statistical analysis comparing the highest dose group with control showed a significant increase in content in all tissues sampled and a significant increase in tissue/cerebellum ratios for thalamus, striatum, and colliculi (all tissues with a high $\mu$ site density). In no tissue, for any of the doses used, was there the expected decrease in specific binding.

**Discussion**

Using $[^{11}C]$diprenorphine PET to quantify opioid receptors, we have shown that, over a range of clinically effective doses of methadone, no significant occupancy of opioid receptors in the brain was detectable and no difference in $[^{11}C]$diprenorphine binding between these patients and healthy controls was observed. Parallel animal studies using an acute analgesic dose of methadone (0.35 mg kg$^{-1}$) similarly revealed no detectable reduction in opioid receptor availability. These findings contrast with our previous demonstration that patients on similar doses of methadone showed dose related blockade of opioid agonist effects (Melichar et al., 2003a).

Our finding contrasts with other recent neuroimaging studies in opioid-dependent subjects. Kling et al. (2000) explored the dose-occupancy relationship of methadone using $[^{18}F]$cyclofoxy PET as a marker of $\mu$ and $\kappa$ opioid receptors in patients scanned just before their next methadone dose (30–90 mg daily). This is similar in dose range to our study, although opposite in timing, as they looked at trough levels of methadone. They found a significant (19–32%) reduction in $[^{18}F]$cyclofoxy binding throughout the brain, particularly in the thalamus, caudate, anterior cingulate, middle temporal, and medial frontal cortices. However, within these areas, only in caudate was there a significant relationship between plasma methadone levels and reduction in $[^{18}F]$cyclofoxy binding.

Another PET radiotracer, $[^{11}C]$carfentanil, a high-affinity $\mu$ opioid receptor agonist, has also been used to examine availability of opioid receptor in patients taking buprenorphine, a $\mu$ partial agonist and $\kappa$ antagonist increasingly used in the treatment of opioid dependence. Partial agonists can be used at much higher concentrations than full agonists due to their increased safety, and at high receptor occupancy, act as antagonists to full opioid agonists (Nutt, 1997). Greenwald et al. (2003) reported that buprenorphine dose dependently reduced whole brain $[^{11}C]$carfentanil binding by $41 \pm 8$, $80 \pm 2$, and $84 \pm 2$ at 2, 16, and 32 mg, respectively. Importantly, this study also demonstrated a direct relationship for buprenorphine between in vivo human brain receptor binding and clinical symptoms. In addition, these reductions were associated with reduced effects of hydromorphone, similar to those reported previously by our group with methadone (Melichar et al., 2003a). So, unlike methadone, buprenorphine at clinical doses does seem to significantly occupy opioid receptors, resulting in almost complete blockade at high doses (16 and 32 mg).

There are several factors that need to be examined to help explain our findings of a lack of occupancy of brain opioid receptors by methadone at clinically effective doses using $[^{11}C]$diprenorphine PET. Does this reflect the binding properties of the opioid agonist methadone, is it an insensitivity of $[^{11}C]$diprenorphine PET or is it a function of the opioid-dependent subjects studied?

With regard to the opioid agonist used, the range of methadone doses prescribed corresponds to those in the study by Kling et al. (2000). In addition, patients in the present study had been stable on their dose for at least 1 month (range 1 month–8 years) with no on-top heroin use, implying that their dose was “clinically effective”. In our study, we only measured the active (R)-enantiomer 4 h after methadone ingestion, whereas Kling (ibid) reported plasma methadone levels 22 h after ingestion. This makes direct comparison of plasma doses in these two studies difficult due to the complex metabolism of methadone (Eap et al., 2002). Plasma methadone levels are related to cerebrospinal fluid (CSF) levels, with CSF levels being $\sim20\%$ of plasma levels (Rubenstein et al., 1978). There is some variability, some of which could be explained by the polymorphic expression of P-glycoprotein: recent work looking at the blood-brain barrier has shown that the entry of methadone is as a substrate of P-glycoprotein (Wang et al., 2004). One can, even with this potential variability, estimate that, in our sample, given plasma levels...
of 176–540 nM, the concentration of methadone in CSF available at the μ opioid receptor is ~40 to 100 nM (~20%). Since the $K_i$ value of methadone is 10.1 nM at the μ opioid receptor (Li et al., 1998), this would suggest that the concentration of methadone is sufficient to occupy a significant proportion of brain μ opioid receptors. Therefore, it is likely that the range of plasma and brain methadone concentrations in the present study are within the same range as in Kling et al. (2000), predicting a reduction in [11C]diprenorphine binding similar to that seen with [13F]cylofoxy.

We used [11C]diprenorphine as a tracer to measure opioid receptor binding. It is a nonselective tracer that labels μ, κ, and δ opioid receptors with similar high affinity (Lewis and Husbands, 2004). We have previously shown that the nonselective opioid antagonist naloxone displaces ~50% of [11C]diprenorphine signal at clinically relevant doses (~13 μg/kg) (Melichar et al., 2003b). In addition, in vivo in human [11C]diprenorphine does seem to be sensitive to increased levels of endogenous opioids since reduced binding has been shown in response to pain (Jones et al., 1991), reading epilepsy (Koepp et al., 1998), and, in animals, to cocaine and ethanol (Gerrits et al., 1999). However, results from our animal study showed that a single high dose of methadone did not reduce specific [11C]diprenorphine binding, whereas previously we have observed that naloxone (Rajeswaran et al., 1991) and buprenorphine (S. P. Hume, unpublished observations) did reduce [123I]diprenorphine binding in the rat brain. Together, these data suggest that interactions between an agonist, partial agonist, or antagonist and the opioid receptor result in differential ability to displace [123I]diprenorphine, perhaps reflecting slightly different interactions with the pharmacophore.

Methadone is a high-efficacy agonist so requires only a small proportion of receptors to exert its effect, and it may be that this low occupation is at the limits of detection using [11C]diprenorphine PET. Methadone has affinity for all three receptor subtypes, but its $K_i$ value at the μ receptor subtype is 10.1 nM (Li et al., 1998) compared with over 1000 nM for $K_i$ for the δ receptor subtype (Raynor et al., 1994). Even in the thalamus where the μ subtype predominates (Cross et al., 1987), no reduction in [11C]diprenorphine binding was seen.
An alternative explanation is the possible internalization of opioid receptors secondary to agonist (methadone) exposure (Borgland et al., 2003) and the ability of the different PET radiotracers to label these internalized receptors. $^{[11]}$C)diprenorphine has been shown to label, in vitro, internalized opioid receptors (Shapira et al. 2001), which could explain the lack of reduction in $^{[11]}$C)diprenorphine binding, whereas data are lacking for the two other tracers.

Another consideration is whether chronic methadone administration might have increased the expression of opioid receptors so obscuring any observable effect of methadone occupation of receptors. In animal, chronic opioid exposure with morphine has been reported to increase receptor number (Zadina et al., 1995). However, a post-mortem study of heroin addicts revealed no difference in opioid receptor levels (Gabilondo et al., 1994). We have preliminary data showing a global increase in $^{[11]}$C)diprenorphine binding in recently detoxified methadone patients (Daglish et al., 2000), which may suggest that methadone does up-regulate receptor number.

In conclusion, we have shown that over a range of clinically effective doses, methadone does not seem to result in significant occupancy of brain opioid receptors. In contrast, in a similar population of methadone maintained opioid-dependent patients, we showed tolerance to opioids with attenuation of objective and subjective responses to hydromorphone. We therefore suggest that clinically relevant doses of methadone result in low ($<10\%$) levels of occupancy that are below the limits of detection with $^{[11]}$C)diprenorphine PET. Our results support evidence that tolerance to methadone is likely to involve changes other than reducing receptor number.

References


