Increased Locomotor Activity Induced by Heroin in Mice: Pharmacokinetic Demonstration of Heroin Acting as a Prodrug for the Mediator 6-Monoacetylmorphine in Vivo

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ABSTRACT
We investigated the relative importance of heroin and its metabolites in eliciting a behavioral response in mice by studying the relationship between concentrations of heroin, 6-monoacetylmorphine (6MAM), and morphine in brain tissue and the effects on locomotor activity. Low doses (subcutaneous) of heroin (≤5 μmol/kg) or 6MAM (≤15 μmol/kg) made the mice run significantly more than mice given equimolar doses of morphine. There were no differences in the response between heroin and 6MAM, although we observed a shift to the left of the dose-response curve for the maximal response of heroin. The behavioral responses were abolished by pretreatment with 1 mg/kg naltrexone. Heroin was detected in brain tissue after injection, but the levels were low and its presence too short-lived to be responsible for the behavioral response observed. The concentration of 6MAM in brain tissue increased shortly after administration of both heroin and 6MAM and the concentration changes during the first hour roughly reflected the changes in locomotor activity. Both the maximal and the total concentration of 6MAM were higher after administration of heroin than after administration of 6MAM itself. The morphine concentration increased slowly after injection and could not explain the immediate behavioral response. In summary, the locomotor activity response after injection of heroin was mediated by 6MAM, which increased shortly after administration. Heroin acted as an effective prodrug. The concentration of morphine was too low to stimulate the immediate response observed but might have an effect on the later part of the heroin-induced behavioral response curve.

Heroin is the most widely abused opioid in the world. It is much more potent than morphine in producing euphoria and pain relief in humans (Kaiko et al., 1981), and reinforcing effects in animals (van Ree et al., 1978; Hubner and Kornetsky, 1992). Heroin has also been shown to be more potent and efficacious as an analgesic agent in rodent pain models (Umans and Inturrisi, 1981; Martin et al., 1998). The pharmacological differences between heroin and morphine could be related to higher efficacy of μ-opioid receptor activation by heroin. However, receptor binding studies have shown that heroin has low affinity for opiate receptors (Inturrisi et al., 1983; Gianutsos et al., 1986). It has therefore been suggested that heroin instead acts as an effective prodrug that mediates its biological effect through its metabolites (Inturrisi et al., 1983) and that the differences in potency are due to pharmacokinetic factors (Oldendorf et al., 1972).

After administration, heroin is rapidly metabolized by sequential deacetylation to 6-monoacetylmorphine (6MAM) and morphine by different tissues in the body, including the brain and blood [t 1/2 (heroin) = 1.3–8 min; t 1/2 (6MAM) = 3–52 min; for overview, see Rook et al., 2006]. In humans, morphine is in turn biotransformed by glucuronidation to pharmacologically active morphine-6-glucuronide (M6G) and inactive morphine-3-glucuronide (M3G) (Glare and Walsh, 1991; Milne et al., 1996). In rats and mice, no or only trace amounts of M6G are produced (Zuccaro et al., 1997; Handal et al., 2002).

Because morphine has been thought to be the major active metabolite after heroin exposure, many of the studies published have used this drug to mimic the effect of heroin. However, 6MAM and M6G are also pharmacologically active (Umans and Inturrisi, 1981; Hubner and Kornetsky, 1992; Handal et al., 2002), and 6MAM may be more effective than morphine in activating μ-opioid receptors (Selley et al., 2001). Umans and Inturrisi (1982) found that blood concentrations of 6MAM or morphine 5 min after administration of...
heroin (10 mg/kg s.c.) were similar to the concentrations achieved after the same dose of 6MAM, but they could not detect heroin itself. Because of the striking similarities in the dose-response and time-action curves between heroin and 6MAM in the analgesic dose range, the same authors have suggested that the behavioral response of heroin is mediated by 6MAM (Umans and Inturrisi, 1981, 1982). Hubner and Kornetsky (1992) also found that 6MAM and heroin were approximately equipotent in affecting both rewarding and aversive brain stimulation. However, none of these earlier studies have confirmed their observations with pharmacokinetic analysis in brain tissue. Therefore, we cannot rule out the possibility that heroin may have an effect on its own.

Locomotor activation is an acute behavioral response to opiate administration in mice (Mørland et al., 1994). This behavior has been linked to opiate receptor stimulation and is completely inhibited by opiate antagonists (Joyce and Iversen, 1979).

The purpose of our study was therefore to compare the onset of a locomotor stimulation with the pharmacokinetic profile of heroin and its metabolites. We administered heroin, 6MAM, and morphine to mice and studied the stimulatory effect on locomotor activity in relation to the time course and concentration of the drugs and their metabolites in blood and brain tissue. This combination of in vivo behavioral studies and opioid analysis in blood and brain tissue allowed us to analyze the possible contribution of the different metabolites to the behavioral response.

Materials and Methods

Animals. Male C57BL/6J-Bom mice (462; 7–8 weeks old; 20–25 g) from Taconic (Bomholt, Denmark) were used in these experiments. The mice were housed seven to eight per cage in the animal facilities at the Norwegian Institute of Public Health (22 ± 1°C; 12:12-h light/dark schedule; light period 7:00 AM–7:00 PM). The mice arrived at least 5 days before the experiments. Commercial mouse pellets and water were available ad libitum. The experimental protocol of this study was approved by the Norwegian Review Committee for the Use of Animal Subjects.

Materials. Heroin hydrochloride (3,6-diacylmorphine; mol. wt. 424) and 6MAM hydrochloride (mol. wt. 419.7) were purchased from Lipomed AG (Arlesheim, Switzerland), morphine hydrochloride (mol. wt. 375.9) was from Norsk Medisinaldepot AS (Oslo, Norway), and naltrexone hydrochloride (mol. wt. 377.9) was from Sigma-Aldrich (Oslo, Norway). The drugs were dissolved in 0.9% saline the same day as they were used. The injections were given subcutaneously, with the exception of naltrexone that was administered intraperitoneally in total volumes of 0.1 ml/10 g mouse.

Locomotor Activity. Locomotor activity was tested individually in chambers using the VersaMax optical animal activity monitoring system (AccuScan Instruments, Inc., Columbus, OH). The 40- × 40-cm chambers, equipped with infrared beams (spaced at 2.5-cm intervals), were divided into four 20- × 20-cm equal quadrants by two perpendicular walls. Two animals were tested simultaneously in each chamber, using two nonadjacent quadrants. Before injections, each animal was individually placed in the activity chamber for 90 min. Thereafter, the mice were gently removed and injected with heroin (1.25–50 μmol/kg; n = 5–7), 6MAM (1.25–50 μmol/kg; n = 4–8), morphine (5–80 μmol/kg; n = 4–6), or saline (n = 4) in another room. Immediately after injection, each mouse was gently returned to its respective activity chamber, and the locomotor activity was measured for another 5 h. In a separate experiment, following the same protocol, naltrexone (0.1 and 1 mg/kg) or saline was injected 15 min before the injection of heroin (15 μmol/kg; n = 3–5), 6MAM (15 μmol/kg; n = 3–4), or morphine (15 or 50 μmol/kg; n = 3–6). We chose the distance traveled in a defined time period as an expression of locomotor activity because previous studies have shown this to be effective (Grung et al., 1998).

Pharmacokinetic Studies. Each mouse was given a bolus injection (5 or 15 μmol/kg s.c.) of heroin, 6MAM, or morphine. The injections were given in total volumes of 0.1 ml/10 g mouse. At given times, the mice (n = 4–8 at each time point) were CO2-anesthetized before blood samples (500 μl) were obtained by heart puncture using a syringe containing 80 μl of sodium fluoride (final concentration, 4 mg/ml) dissolved in heparin (100 IU/ml). Sodium fluoride was used to inhibit the plasma esterase activity, thereby stabilizing the amount of heroin and 6MAM (Brogan et al., 1992). The blood was transferred to a microcentrifuge tube; diluted 1:1 in ice-cold 5 mM ammonium formate buffer, pH 3.1; and immediately frozen in liquid N2. After blood sampling, the brain (except cerebellum) was quickly removed; washed in ice-cold 5 mM ammonium formate buffer, pH 3.1; blotted on a filter paper; and homogenized (0.35 g tissue/ml homogenate) in ice-cold 5 mM ammonium formate buffer, pH 3.1, before being frozen in liquid N2. Ice-cold acidic buffer was used to dilute the blood samples and to homogenize brain tissue because heroin is most stable at low temperatures and low pH (Barrett et al., 1992). All samples were stored at −80°C until analyzed. To counteract the poor stability of heroin, samples from mice injected with heroin were analyzed within 1 to 2 days, whereas samples from mice injected with 6MAM, morphine, or saline were analyzed within 1 week.

LC-MS/MS Analysis. LC-MS/MS analysis was performed as described in detail (R. Karinen, J. M. Anderson, Å. Ripel, I. Hasvold, A. B. Hopen, J. Mørland, and A. S. Christophersen, submitted for publication). We added 50 μl of internal standard (0.5 μM) and 500 μl of ice-cold acetonitrile/methanol (85:15) to 200 μl of the samples, and these samples were shaken immediately. The samples were frozen for at least 10 min, thawed on ice, and centrifuged at 4500 rpm (3900g at 4°C for 10 min). The organic phase was transferred to a glass tube and evaporated to dryness at 40°C under a gentle stream of nitrogen. The dry residue was then reconstituted with 100 μl of ice-cold mobile phase (3% acetonitrile/97% 5 mM ammonium formate buffer, pH 3.1), centrifuged, and transferred to autosampler vials.

The opiates were separated at 35°C on a XTerra MS C18 column (Waters, Milford, MA) using gradient elution with mobile phase consisting of acetonitrile and 5 mM ammonium formate buffer, pH 3.1. Flow rate was set to 0.2 ml/min. A triple quadrupole LC-MS/MS system (Micromass MS Technologies, Waters) equipped with an electron ion spray source operated in the positive ionization and multiple reaction monitoring mode was used for quantification. The calibration standards for heroin were handled separately from the metabolites to investigate the stability of heroin during sample preparation and analysis. The limits of detection in blood were 0.0065 mg/l for M3G, 0.00060 mg/l for M6G, 0.00049 mg/l for morphine, 0.00033 mg/l for heroin, and 0.00096 mg/l for heroin. Limits of detection in brain tissue were 0.020 μg/g for M3G, 0.0018 μg/g for M6G, 0.00033 mg/g for morphine, 0.0010 μg/g for heroin, and 0.0029 μg/g for heroin. The interassay variability was <15% for all compounds.

Data Analysis. Because the amount of blood needed for the LC-MS/MS analysis did not allow us to take serial blood samples from each mouse, we used mean blood concentration from four to eight animals per time point to draw the concentration-time curves. Data are presented as mean ± S.E.M. unless otherwise stated. Statistical analysis of the locomotion data were carried out by General Linear Model for repeated measures with drug and dose as fixed factors and time as repeated factor. The Greenhouse-Geisser correction of the degrees of freedom was used when sphericity was violated. The pharmacokinetic data were analyzed with univariate analysis of variance with drug as factor. All differences between groups were analyzed with one-way analysis of variance. Scheffe test was used as the post hoc test (SPSS, version 14.0; SPSS Inc., Chicago, IL). p values less than 0.05 are considered as statistically significant.
Results

Locomotor Activity. The different drugs \( F(2,107) = 9.602; p < 0.001 \) and doses \( F(8,107) = 68.692; p < 0.001 \) administered as well as their interaction \( \text{drug} \times \text{dose}; F(11,107) = 13.362; p < 0.001 \) had a significant effect on locomotor activity. Their effect was also dependent on time \( \text{drug} \times \text{dose} \times \text{time}; F(50,485) = 6.761; p < 0.001 \) (Fig. 1). The locomotor effect declined to zero after 3 to 4 h. Injection of saline did not induce changes in locomotor activity in the mice (data not shown).

The total distance traveled was related to drug doses. At low doses (<15–20 \( \mu \text{mol/kg} \)), heroin and 6MAM stimulated the locomotor activity dose-dependently, whereas higher doses resulted in a plateau for the total distance traveled. No differences were shown between heroin and 6MAM (Fig. 2, top).

Concerning \( E_{\text{max}} \), administration of heroin and 6MAM showed inverted U-shaped dose-effect curves. Injection of low doses stimulated \( E_{\text{max}} \) dose-dependently. The maximum was reached with 5 \( \mu \text{mol/kg} \) heroin and 15 \( \mu \text{mol/kg} \) 6MAM, whereas higher doses resulted in lower \( E_{\text{max}} \) (Fig. 2, bottom). Compared with 6MAM, the dose-\( E_{\text{max}} \) relationship for heroin seemed to be shifted to the left, but there were no statistically significant differences in \( E_{\text{max}} \) between heroin and 6MAM.

After high doses of heroin and 6MAM, a toxic or narcotic effect was clearly seen in that mice showed staggering and incoherent running, reducing the maximal distance run. No consistent movement patterns could be monitored by the activity recording instrument.

For the doses tested, morphine showed increasing dose-response curves for both \( E_{\text{max}} \) and the total run distance. Compared with heroin and 6MAM, both these measures were different \( (p < 0.001) \), showing less stimulatory potential at low doses (<30 \( \mu \text{mol/kg} \)) and stronger potential at high doses for morphine (Fig. 2).

The high dose of naltrexone (1 mg/kg) reduced all locomotor activity induced by heroin, 6MAM, and morphine to con-
trol levels. In addition, the low dose (0.1 mg/kg) caused a strong reduction in the opioid-stimulated activity, which was of similar magnitude for all drugs and doses tested (Fig. 3).

**Pharmacokinetic Studies.** The blood and brain concentrations of heroin, 6MAM, morphine, and M3G measured 1 to 120 min after equimolar injections (subcutaneous) of 5 and 15 μmol/kg heroin, 6MAM, and morphine, respectively, are shown in Figs. 4 and 5. The concentration profiles of heroin, 6MAM, and morphine in brain reflected the profiles seen in blood. However, the maximum concentrations were reached later in the brain compared with blood. M3G was abundant in the blood but was hardly detectable in brain tissue. Heroin was only detected in mice injected with heroin. M6G was not detected in any of the samples.

Administration of heroin showed maximal concentrations of the drug in blood and brain tissue 1 and 3 min after injection of 5 and 15 μmol/kg, respectively. However, both the maximal (C_max) and the total (AUC) concentrations were low, and the drug disappeared quickly (Figs. 4 and 5; Tables 1 and 2). Injection of 15 μmol/kg heroin gave 6MAM concentrations in both blood and brain tissue 1 and 3 min after injection of 5 and 15 μmol/kg heroin, 6MAM, and morphine, respectively, that were approximately twice the concentrations seen after injection of 6MAM itself (Fig. 5; Table 2). Maximal brain concentrations of 6MAM were achieved 10 and 15 min after injection of heroin and 6MAM, respectively, and they were significantly different from each other [F(1,10) = 10.994; p < 0.01] (Table 1). Injection of the low dose (5 μmol/kg) of heroin gave higher 6MAM concentrations in the brain compared with injection of 6MAM 15 min [F(1,72) = 11.080; p < 0.001] (Fig. 4), with maximal concentrations for both drugs at 15 min [F(1,6) = 12.072; p < 0.05] (Table 1). The difference in the C_max values in blood was not statistically significant.

Although the blood concentrations of morphine were higher after morphine administration than after heroin and 6MAM administration [5 μmol/kg: F(2,98) = 72.113; p < 0.001; 15 μmol/kg: F(2,115) = 43.364; p < 0.001] (Figs. 4 and 5), injection of morphine resulted in lower brain concentrations of the compound compared with injection of heroin [5 μmol/kg: F(2,102) = 16.553; p < 0.02; 15 μmol/kg: F(2,122) = 27.626; p < 0.001] (Figs. 4 and 5). Both doses of heroin and 6MAM led to significantly higher brain concentrations of morphine than those of heroin [F(1,10) = 12.072; p < 0.001] (Figs. 4 and 5). 6MAM concentrations in the brain compared with injection of 6MAM itself (Fig. 5; Table 2). Maximal brain concentrations of 6MAM were achieved 10 and 15 min after injection of heroin and 6MAM, respectively, and they were significantly different from each other [F(1,10) = 10.994; p < 0.01] (Table 1). Injection of the low dose (5 μmol/kg) of heroin gave higher 6MAM concentrations in the brain compared with injection of 6MAM 15 min [F(1,72) = 11.080; p < 0.001] (Fig. 4), with maximal concentrations for both drugs at 15 min [F(1,6) = 12.072; p < 0.05] (Table 1). The difference in the C_max values in blood was not statistically significant.

**Discussion**

In this study, we investigated the effect of heroin, 6MAM, and morphine on locomotor activity in mice and the presence of these drugs in brain tissue to examine the relative importance of the different compounds in eliciting a behavioral response. Administration of equivalent doses of heroin, 6MAM, and morphine showed strikingly different potential to affect locomotor activity, which in mice reflects the presence of active compounds in the brain (Mørland et al., 1994; Handal et al., 2002). Low doses of heroin (±5 μmol/kg) or 6MAM (±15 μmol/kg) made the mice run significantly more compared with mice given equimolar doses of morphine, indicating differences in the stimulating potential between the drugs. After high doses of heroin and 6MAM, a toxic or narcotic effect was clearly seen, resulting in staggering and incoherent running, reducing the maximum distance run. Administration of high doses of morphine did not show any pronounced sedative or narcotic effects, although the highest dose tended to reduce the maximum distance run. A morphine response comparable with the maximal responses of heroin and 6MAM was achieved at 50 μmol/kg. That a high morphine dose is needed to induce a behavioral response has been demonstrated previously (Grung et al., 1998; Handal et al., 2002). On the whole, our findings are in agreement with previous work showing that heroin is more potent than morphine (Umans and Inturrisi, 1981; Hubner and Kornetsky, 1992).

Absence of a significant difference in the response between heroin and 6MAM could indicate that hydrolysis of heroin precedes the pharmacological effect and that 6MAM is the main mediator after a heroin injection. On the other hand, although the shift to the left of the dose-E_max curve for heroin compared with 6MAM was not statistically significant, it might indicate a potency difference between the drugs. A higher potency might explain that both the maximum effect and the stultifying effects of heroin occurred at a lower dose compared with 6MAM. Thus, we looked at the pharmacokinetics of heroin, 6MAM, and morphine in brain tissue for the doses that led to maximum locomotor activity after heroin and 6MAM administration. The findings in brain were compared with the presence of the compounds in blood to clarify the transport across the blood-brain barrier (BBB).

Heroin was detected in both brain and blood. However, the concentrations were extremely low, and the drug disappeared shortly after administration, supporting previous findings that heroin is rapidly deacetylated in the body (Inturrisi et al., 1984; Gyr et al., 2000). The biotransformation may take place by different esterases in blood, brain, and other tissues (Kamendulis et al., 1996; Salmon et al., 1999; Rook et al., 2006), but heroin may also undergo nonenzymatic degradation to 6MAM (Selley et al., 2001).
Both the maximal and the total concentrations of 6-MAM in brain tissue were higher after administration of heroin than after administration of 6-MAM itself. For the 15-mmol/kg dose, the blood concentrations of 6-MAM were also higher after injection of heroin. Because the drugs were given as equimolar doses, this implies that heroin was probably absorbed more effectively than 6-MAM from the injection site and quickly deacetylated to 6-MAM. This is in accordance with the work of Umans and Inturrisi (1981, 1982) and Lockridge et al. (1980), showing that much of the

![Fig. 4. Concentrations of heroin, 6-MAM, morphine, and M3G in blood (micromolar) and brain tissue (nanomoles per gram) as function of time (minutes) after injection (subcutaneous) of 5 μmol/kg heroin, 6-MAM, and morphine. Values are mean ± S.E.M., n = 4 to 8.](image-url)
hydrolysis of heroin to 6MAM occurs at the subcutaneous injection site and in the blood.

Heroin and 6MAM are both transported in blood and pass the BBB easily (Oldendorf et al., 1972; Umans and Inturrisi, 1981). We found that the presence of heroin and 6MAM in brain tissue correlated well with the presence in blood, indicating effective uptake of these substances through the BBB. Morphine, in contrast, showed a delayed uptake into the brain. For M3G, high concentrations were found in blood but only trace amounts were seen in the brain, indicating a restricted transport through the BBB. However, this metabolite does not stimulate μ-opioid receptors (Lambert et al., 1993; Löser et al., 1996), and its possible pharmacological action is still under consideration.

In Fig. 6, the behavior (Figs. 1 and 2) and the pharmacokinetics of the drugs (Figs. 4 and 5) are compared. For the 5-μmol/kg dose, the total locomotor activity induced by heroin or 6MAM was identical and corresponded neatly with the

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**Fig. 5.** Concentrations of heroin, 6MAM, morphine, and M3G in blood (micromolar) and brain tissue (nanomoles per gram) as function of time (minutes) after injection (subcutaneous) of 15 μmol/kg heroin, 6MAM, and morphine. Values are mean ± S.E.M., n = 4 to 7.
affinity for that the presence of heroin, which was short-lived, has low administration of both heroin and 6MAM. It seems unlikely brain concentration curves of 6MAM, which rose shortly after Values are calculated as AUC from the curve for the mean values of each time point. The range is shown in parentheses Total brain concentration versus time (AUC) of drugs and metabolites after injection (subcutaneous) of 5 and 15 μmol/kg heroin, 6MAM, and morphine Values are mean ± S.E.M., n = 4 to 8 mice.

<table>
<thead>
<tr>
<th>Injected Drug</th>
<th>Heroin</th>
<th>6MAM</th>
<th>Morphine</th>
<th>M3G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heroin, 5 μmol/kg</td>
<td>0.03 ± 0.019</td>
<td>0.83 ± 0.038†</td>
<td>0.20 ± 0.025‡</td>
<td>N.D.</td>
</tr>
<tr>
<td>Heroin, 15 μmol/kg</td>
<td>0.21 ± 0.051</td>
<td>4.70 ± 0.706‡</td>
<td>0.73 ± 0.028†</td>
<td>0.02 ± 0.011</td>
</tr>
<tr>
<td>6MAM, 5 μmol/kg</td>
<td>0.61 ± 0.049</td>
<td>2.76 ± 0.203</td>
<td>0.20 ± 0.013†</td>
<td>N.D.</td>
</tr>
<tr>
<td>6MAM, 15 μmol/kg</td>
<td>2.26 ± 0.203</td>
<td>0.46 ± 0.056</td>
<td>0.07 ± 0.052</td>
<td></td>
</tr>
<tr>
<td>Morphine, 5 μmol/kg</td>
<td>0.11 ± 0.005</td>
<td>N.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine, 15 μmol/kg</td>
<td>0.45 ± 0.021</td>
<td>0.07 ± 0.018</td>
<td></td>
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</tbody>
</table>

N.D., not determined.

† Significant difference compared with 6MAM injected, F(1,10) = 12.072; p < 0.05.
‡ Significant difference compared with 6MAM injected, F(1,10) = 10.994; p < 0.01.
§ Significant difference compared with morphine injected, F(2,12) = 17.577; p < 0.001.

TABLE 2
Total brain concentration versus time (AUC) of drugs and metabolites after injection (subcutaneous) of 5 and 15 μmol/kg heroin, 6MAM, and morphine Values are calculated as AUC from the curve for the mean values of each time point. The range is shown in parentheses [AUC\text{max}(AUC – S.E.M.) – AUC\text{max}(AUC + S.E.M.)], n = 4 to 8 mice.

<table>
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<th>6MAM</th>
<th>Morphine</th>
<th>M3G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heroin, 5 μmol/kg</td>
<td>0.05 (0.03–0.07)</td>
<td>2.99 (2.76–3.22)</td>
<td>0.84 (0.72–0.96)</td>
<td>N.D.</td>
</tr>
<tr>
<td>Heroin, 15 μmol/kg</td>
<td>0.53 (0.41–0.65)</td>
<td>17.68 (15.35–20.00)</td>
<td>3.53 (2.92–4.14)</td>
<td>0.051 (0.018–0.085)</td>
</tr>
<tr>
<td>6MAM, 5 μmol/kg</td>
<td>2.38 (2.07–2.69)</td>
<td>0.73 (0.67–0.79)</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>6MAM, 15 μmol/kg</td>
<td>8.69 (7.32–10.06)</td>
<td>1.92 (1.65–2.19)</td>
<td>0.147 (0.062–0.232)</td>
<td></td>
</tr>
<tr>
<td>Morphine, 5 μmol/kg</td>
<td>0.56 (0.49–0.63)</td>
<td>N.D.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine, 15 μmol/kg</td>
<td>2.07 (1.88–2.25)</td>
<td>0.22 (0.15–0.29)</td>
<td></td>
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N.D., not determined.

brain concentration curves of 6MAM, which rose shortly after administration of both heroin and 6MAM. It seems unlikely that the presence of heroin, which was short-lived, has low affinity for μ-opioid receptors, and was hardly detectable, could be responsible for the behavioral response observed. This supports the idea of heroin acting as a prodrug. As can be seen, the morphine concentrations achieved were too low to stimulate locomotor activity.

With the 15 μmol/kg dose, there was a good correspondence between locomotor activity and brain concentrations of 6MAM in mice injected with 6MAM. In mice administered with heroin, both an immediate behavioral response and a quick and substantial increase in the presence of 6MAM were seen. However, the maximal locomotor activity was clearly reduced and delayed compared with the maximal activity observed after administration of 6MAM. The explanation for this may be that the 6MAM concentration was approximately twice as high after injection of this heroin dose compared with injection of the equimolar dose of 6MAM and therefore induced stultifying effects. The slow increase in the morphine concentration could not explain the immediate behavioral response. However, the morphine concentrations achieved were able to induce locomotor activity and were more long-lasting than the presence of 6MAM. It therefore seems reasonable to assume that morphine might influence the prolonged activity seen after administration of heroin.

As far as we are aware, this article is the first to show a direct relationship between a behavioral response and the presence of the different metabolites after injection of heroin. Our findings do not only support earlier suggestions, based on behavioral studies, that 6MAM contributes to the pharmacological activity of heroin (Umans and Inturrisi, 1981; Hubner and Kornetsky, 1992) but demonstrate that there is a correlation between locomotor activity after administration of heroin and the presence of 6MAM in brain, clearly indicating that 6MAM is the active drug causing this behavior and that heroin is acting as a prodrug. The other important finding, which can be deduced from the comparisons of brain drug concentrations and the effects observed, is that morphine derived from heroin or 6MAM plays no major role in the immediate heroin response.

It has been reported that both μ-opioid receptor-1-deficient CXBK mice and μ-opioid receptor-1 knockout mice respond quite poorly to the analgesic effect of morphine, whereas their sensitivity to heroin and 6MAM is normal (Rossi et al., 1996; Schuller et al., 1999). It has also been shown that the μ-opioid receptor antagonist 3-methoxynaltrexone blocks the heroin and 6MAM analgesia in rodents but is ineffective against morphine (Brown et al., 1997). These findings suggest that heroin/6MAM may act through a subreceptor or via a binding site different from morphine. We used naltrexone to show that the increase in locomotor activity was mediated via opioid receptors without major differences between the three opioids.

The much higher brain concentrations of 6MAM compared with morphine after administration of heroin or 6MAM may be because of restricted deacetylation of 6MAM both in blood and brain (Salmon et al., 1999). In addition, poor uptake of morphine through the BBB may play a role (Oldendorf et al., 1972).

We found that injection of both doses of heroin resulted in brain morphine concentrations that were 1.5 to 1.7 times the concentrations achieved after injection of morphine itself.
This is in accordance with the findings of Way et al. (1965) who detected higher brain morphine levels after administration of heroin than after a comparable dose of morphine. This supports the idea of heroin acting as an effective transport form. In blood, we found the highest morphine concentrations after injection of morphine itself, which differs from the findings of Pacifici et al. (2000) who reported nearly overlapping serum profiles of morphine after injection of heroin and morphine. However, they were not able to detect heroin and found only minor amounts of 6MAM. A possible explanation may be that these authors did not use sodium fluoride or acidic buffer to inhibit esterases; therefore, the morphine measured could be a result of in vitro deacetylation of heroin and 6MAM after sampling.

In conclusion, our study shows that subcutaneous administration of heroin and 6MAM induces a quick and substantial increase in locomotor activity in mice. From its known pharmacological properties, and its low concentration and rapid disappearance in the brain, heroin itself seems to have no or very little biological activity per se. The immediate heroin response seems to be mediated by 6MAM, which becomes present at high concentrations in brain tissue shortly after heroin administration. 6MAM also has high efficiency at μ-opioid receptors. The brain concentrations of morphine are too low to participate in the immediate response of heroin but may contribute to the later effects observed after heroin and 6MAM administration.
6-Monoacetylmorphine Mediates the Heroin Response in Vivo

References


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