

## RESEARCH PAPER

# Levels of heroin and its metabolites in blood and brain extracellular fluid after i.v. heroin administration to freely moving rats

A Gottås, E L Øiestad, F Boix, V Vindenes, Å Ripel, C H Thaulow and J Mørland

*Department of Drug Abuse Research and Method Development, Division of Forensic Medicine and Drug Abuse Research, Norwegian Institute of Public Health, Oslo, Norway*

### Correspondence

André Gottås, Department of Drug Abuse Research and Method Development, Norwegian Institute of Public Health, Pb 4404 Nydalen, 0403 Oslo, Norway. E-mail: andre.gottas@fhi.no

### Keywords

microdialysis; pharmacokinetic; prodrug; metabolite kinetic; CNS; distribution; opioid; heroin; morphine; acetylmorphine

### Received

9 April 2013

### Revised

24 June 2013

### Accepted

1 July 2013

## BACKGROUND AND PURPOSE

Heroin, with low affinity for  $\mu$ -opioid receptors, has been considered to act as a prodrug. In order to study the pharmacokinetics of heroin and its active metabolites after i.v. administration, we gave a bolus injection of heroin to rats and measured the concentration of heroin and its metabolites in blood and brain extracellular fluid (ECF).

## EXPERIMENTAL APPROACH

After an i.v. bolus injection of heroin to freely moving Sprague–Dawley rats, the concentrations of heroin and metabolites in blood samples from the vena jugularis and in microdialysis samples from striatal brain ECF were measured by ultraperformance LC-MS/MS.

## KEY RESULTS

Heroin levels decreased very fast, both in blood and brain ECF, and could not be detected after 18 and 10 min respectively. 6-Monoacetylmorphine (6-MAM) increased very rapidly, reaching its maximal concentrations after 2.0 and 4.3 min, respectively, and falling thereafter. Morphine increased very slowly, reaching its maximal levels, which were six times lower than the highest 6-MAM concentrations, after 12.6 and 21.3 min, with a very slow decline during the rest of the experiment and only surpassing 6-MAM levels at least 30 min after injection.

## CONCLUSIONS AND IMPLICATIONS

After an i.v. heroin injection, 6-MAM was the predominant opioid present shortly after injection and during the first 30 min, not only in the blood but also in rat brain ECF. 6-MAM might therefore mediate most of the effects observed shortly after heroin intake, and this finding questions the general assumption that morphine is the main and most important metabolite of heroin.

## Abbreviations

6-MAM, 6-monoacetylmorphine; AUC<sub>Last</sub>, area under the concentration–time curve from time zero to last sample time; C<sub>max</sub>, maximum concentration; BBB, blood–brain barrier; Cl, clearance; C<sub>u</sub>, concentration of unbound analyte in brain ECF; ECF, extracellular fluid; LLOQ, lowest level of quantification; LOD, lowest level of detection; M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide;  $\mu$ M, concentration ( $\mu$ mol·L);  $\mu$ mol, molar units; T<sub>max</sub>, time to reach C<sub>max</sub>; UPLC-MS/MS, ultraperformance LC-MS/MS; V<sub>d</sub>, volume of distribution during the terminal phase

## Introduction

Heroin is considered to be one of the most addicting drugs of abuse (Anthony *et al.*, 1994). It does however have a low-binding affinity (Inturrisi *et al.*, 1983; Gianutsos *et al.*, 1986) and efficacy (Selley *et al.*, 2001) at the  $\mu$ -opioid receptors (MORs), and is believed to act mainly as a prodrug (Inturrisi *et al.*, 1983; Gianutsos *et al.*, 1986). Heroin is highly lipophilic and has a high apparent permeability through the blood-brain barrier (BBB) (Oldendorf *et al.*, 1972). Thus an effective delivery of active metabolites to the brain has been used as an explanation for the highly addictive properties of heroin, where morphine customarily has been considered to be the most relevant active compound mediating heroin euphoria and reward. However, other metabolites, like 6-monoacetylmorphine (6-MAM) and morphine-6-glucuronide (M6G), also possess pharmacological activity (Way *et al.*, 1960; Inturrisi *et al.*, 1983; Pasternak *et al.*, 1987; Strandberg *et al.*, 2006) and are therefore likely to contribute to the heroin effects. The pharmacological contribution of 6-MAM after acute or chronic administration of heroin has however only to a limited extent been studied.

Andersen *et al.* (2009) have shown that 6-MAM reached much higher concentrations than heroin or morphine in both blood and brain after s.c. administration of heroin to mice. Moreover, the acute behavioural effects observed after heroin administration were more closely related to brain concentrations of 6-MAM than to heroin and morphine, indicating that 6-MAM was the compound mainly responsible for these effects. Subsequent pharmacokinetic analysis of these data showed that the transfer rate for heroin from blood to brain was much lower than its conversion rate to 6-MAM in blood. These results would imply that heroin was rapidly metabolized to 6-MAM and only a small fraction of the heroin dose was able to reach the brain, while the high 6-MAM concentrations in brain were merely reflecting transfer of 6-MAM formed in blood (Boix *et al.*, 2013). Together, these observations challenge the view that morphine, formed in the brain from rapidly entering heroin, is the compound mainly responsible for the effects of heroin (Oldendorf *et al.*, 1972; National Institute on Drug Abuse, 2005). Accordingly, they also question the generalization of numerous neurobiological studies using morphine, to also account for mechanisms involved in heroin-related effects.

The above-described observations showing that metabolism of heroin to 6-MAM mainly takes place in blood, before reaching the brain (Andersen *et al.*, 2009; Boix *et al.*, 2013), could yet be particularly related to the s.c. administration route used in those studies. During the passage through the s.c. compartment heroin might be subject to metabolism, influencing the disposition of heroin and its metabolites observed in the blood and, therefore, their pharmacokinetics. Furthermore the absorption from a s.c. depot will give a more gradual presentation of drug to the heroin-metabolizing enzymes in blood, making saturation of their catalytic capacity less likely, allowing more 6-MAM to be formed. These factors would however be reduced by an i.v. bolus administration, also representing the most common, and preferred, route of administration in human abusers. Extravascular routes, like s.c. or i.m., do not seem to provide the quick and powerful rush normally associated with i.v. heroin. The high

bioavailability and fast distribution of the drug achieved through i.v. administration will enhance the transfer of drug to its main effect site/compartment, in the brain for the case of heroin and its metabolites. In the present study, we tested if the distribution of the active heroin metabolites in blood and brain were similar after i.v. administration compared with the observations seen after s.c. injections in mice, where 6-MAM achieves higher levels than heroin or morphine. Further, as the opioid receptors activated by opioids in the brain are faced towards the extracellular fluid (ECF), the unbound brain ECF concentrations of opioids are more relevant than the concentrations measured in whole brain samples including both extracellular and intracellular fluid (Hammarlund-Udenaes *et al.*, 2008). For this purpose, we administered an i.v. bolus of heroin and measured heroin and the three metabolites [6-MAM, morphine and morphine-3-glucuronide (M3G)] by ultraperformance LC-MS/MS (UPLC-MS/MS) in repeated samples from blood and brain ECF, obtained from striatum by microdialysis, in freely moving rats. To our knowledge this has not been studied previously. The literature related to heroin pharmacokinetics, including experimental animals, is sparse. Knowledge of the distribution of heroin and, especially, its active metabolites over time in blood and brain ECF, the compartment considered relevant for the target receptors involved in the euphorogenic and rewarding effects, is important for a proper understanding of the acute effects of heroin.

## Methods

### Chemicals and reagents

Heroin hydrochloride, heroin-d3, heroin-d9, 6-MAM, 6-MAM-d3, 6-MAM-d6, morphine-d3, M6G, M3G and M3G-d3 were obtained from Lipomed (Lipomed GmbH, Arlesheim, Switzerland), morphine from NMD (NMD Grossisthandel AS, Oslo, Norway) and morphine-d6 from Toronto Research Chemicals Inc. (Ontario, Canada). Standard compounds were stored according to supplier recommendations. HPLC-grade methanol and acetonitrile were purchased from Labscan Ltd. (POCH SA, Gliwice, Poland), and analytical grade ammonium formate and formic acid from Merck (Whitehouse Station, NJ, USA). All water used was provided by a MilliQ A10 purification system (Merck KGaA, Darmstadt, Germany). Stock and working solutions were prepared as described previously (Gottas *et al.*, 2012).

### Animals and conditions

Male Sprague-Dawley rats from Taconic (Borup, Denmark), 7–8-week-old and weighing about 300 g at surgery, were used ( $n = 14$ ). They were housed in pairs at standard housing conditions (08:00–20:00 h lights on), with free access to food and water. The surgery was performed at least 4 days after arrival. After implantations of catheters and the guide cannula, each rat was placed in a special individual cage to avoid injury of the rat and the implants.

The experimental protocol was approved by the Norwegian National Animal Research Authority and carried out in accordance to Norwegian regulations and international standards.



**Table 1**

UPLC gradient profile

Time (min)	Methanol mobile phase (%)	Ammonium format mobile phase (%)
0.00	0	100
0.50	10	90
2.60	50	50
3.00	90	10
4.50	10	90
5.10	0	100
5.20	100	0

The method validation and analysis for the blood and brain tissue samples were performed with a Waters Quattro Premier XE MS/MS using the same instrumental parameters as for dialysate (Gottas *et al.*, 2012), with some modifications. The blood UPLC gradient profile is shown in Table 1. Data were processed with the TargetLynx program (Waters, Milford, MA, USA), using peak area for quantification. Calibration curves with five to seven points were applied for the analytes. The lowest level of quantification (LLOQ) and lowest level of detection (LOD) are presented in Table 2. The within-day and between-day precision and accuracy (Table 3) showed a good precision and accuracy for the analytical method. No endogenous interferences were found in blank rat blood samples.

### Microdialysis probe recovery

Recovery for each microdialysis probe and the analytes were calculated by retrodialysis using isotope analogues as

**Table 2**

Molecular weight, limit of detection and lower limit of quantitation with precision and bias

Substance	Molecular weight	LOD		LLOQ		CV (%)	Bias (%)
		nmol·mL <sup>-1</sup>	ng·mL <sup>-1</sup>	nmol·mL <sup>-1</sup>	ng·mL <sup>-1</sup>		
Heroin	369.4	0.0002	0.07	0.008	3.0	14	4
6-MAM	327.4	0.0011	0.36	0.006	2.0	10	-1
Morphine	285.3	0.0009	0.26	0.006	1.7	18	-6
M3G	461.5	0.0005	0.23	0.02	2.2	14	-5

**Table 3**

Added concentrations, within-day precision and accuracy (bias) and between-day precision and accuracy (bias)

Substance	Added concentration		Within day		Between day	
	nmol·mL <sup>-1</sup>	ng·mL <sup>-1</sup>	CV (%)	Bias (%)	CV (%)	Bias (%)
Heroin						
QC (high)	5	1851	3	-9	3	-6
QC (medium)	0.5	185	4	-11	5	-5
QC (low)	0.05	18	4	-9	5	-4
6-MAM						
QC (high)	5	1643	3	-1	5	-1
QC (medium)	0.5	164	2	4	5	0.5
QC (low)	0.05	16	4	10	5	3
Morphine						
QC (high)	5	1427	5	-2	7	0
QC (medium)	0.5	143	6	14	8	3
QC (low)	0.05	14	6	17	12	8
M3G						
QC (high)	4.97	2293	4	-5	12	-6
QC (medium)	0.5	229	8	0	10	-1
QC (low)	0.014	6	11	7	18	-2

described by Gottås *et al.* (2012). This procedure was used for calculating the concentration of unbound analyte ( $C_u$ ) in brain ECF.

The average recovery values ( $\pm$ SD) for the deuterated recovery calibrators were  $28 \pm 12\%$  for heroin-d9,  $22 \pm 9\%$  for 6-MAM-d3,  $21 \pm 10\%$  for morphine-d6 and  $19 \pm 9\%$  for M3G-d3 ( $n = 7$ ,  $n = 3$  for M3G-d3). For samples without recovery estimation, the average recovery was used to calculate  $C_u$ .

### Data analysis

From a total of 14 animals, and due to technical problems with the sampling equipment, it was possible to collect both blood and brain ECF data from five animals: two animals provided only brain ECF data and three animals only blood data. Both sampling methods failed after various time periods in four animals that could supply only full-tissue (homogenate) brain data.

To compensate for samples taken at different time intervals, and the fact that not all animals gave complete data sets, blood and microdialysis sample data were fitted by a population method using the program Kinetica 5.1 (Thermo Fisher Scientific Inc., Waltham, MA, USA). All results were fitted by an extravascular model, except for heroin in blood collected after i.v. administration, which was fitted by an i.v. bolus model. The number of compartments in the model did not have any noteworthy influence on the results, and the model with the lowest Akaike information criteria was selected in order to assure the best fitting for each analyte (Ludden *et al.*, 1994; Glatting *et al.*, 2007).

The calculated fitted results were further used in a non-compartmental analysis to calculate the area under the concentration–time curve from time zero to last sample time ( $AUC_{last}$ ), the estimated maximum concentration ( $C_{max}$ ) and the time to reach  $C_{max}$  ( $T_{max}$ ) for all the different substances. The  $t_{1/2}$  were calculated for both heroin and 6-MAM in blood and brain ECF after taking fitted log concentration versus time plots into account. Additionally, clearance (Cl) and volume of distribution during the terminal phase (Vd) were calculated based on heroin levels measured in blood. Because virtually all heroin is metabolized to 6-MAM without loss into urine (Rook *et al.*, 2006b), the virtual dose of 6-MAM can be considered similar to the actual heroin dose, allowing the calculation of Cl and Vd for 6-MAM based on blood concentrations as well. The AUC calculations were based on a mixed log linear model implemented in the Kinetica software. For heroin in blood an i.v. bolus model was used, whereas an extravascular model was used for the metabolites in blood and all the compounds in brain ECF. The log-transformed concentration versus time plots were used to assess the distribution ( $\alpha$ ) and elimination phase. All data below LLOQ were discarded from the data set, except for M3G in dialysate, where LOD was used as limit for further analysis to avoid too few data points for analysis in Kinetica.

## Results

### Physiological observations

The heroin dose (1.3 mg) chosen in this study was relatively high to ensure measurable concentrations of heroin in the

brain ECF compartment. Immediately after injection the animals showed a complete sedation, with severe muscle rigor and reduced respiration rate. Indication of cyanosis, with change in blood colour and thickening of blood, was observed and made blood sampling challenging.

### Blood pharmacokinetics

The concentration versus time profiles are shown in Figure 2A, and the calculated pharmacokinetic values are listed in Table 4. The heroin level in blood declined below LLOQ already after about 18 min, with a biphasic concentration versus time curve. The  $C_{max}$  for heroin was  $3.8 \mu\text{M}$  as calculated by Kinetica. This value must however be considered as extremely uncertain due to few data points within the distribution phase ( $\alpha$  phase). The initial  $\alpha$  phase (ultra-rapid distribution, between 0 and 1 min) had an apparent  $t_{1/2}$  of at least 0.3 min and the terminal elimination phase (between 2 and 18 min) a  $t_{1/2}$  of 2.7 min. However, the highest measured concentration of heroin from an animal was  $0.95 \mu\text{M}$ , obtained in the first sample taken after 2 min. The corresponding concentration of 6-MAM in the same sample was  $8.86 \mu\text{M}$ , about nine times higher than the heroin concentration. In general, however, the 6-MAM concentrations were about 18 times higher than the heroin concentration in the first blood samples measured. Further, based on the

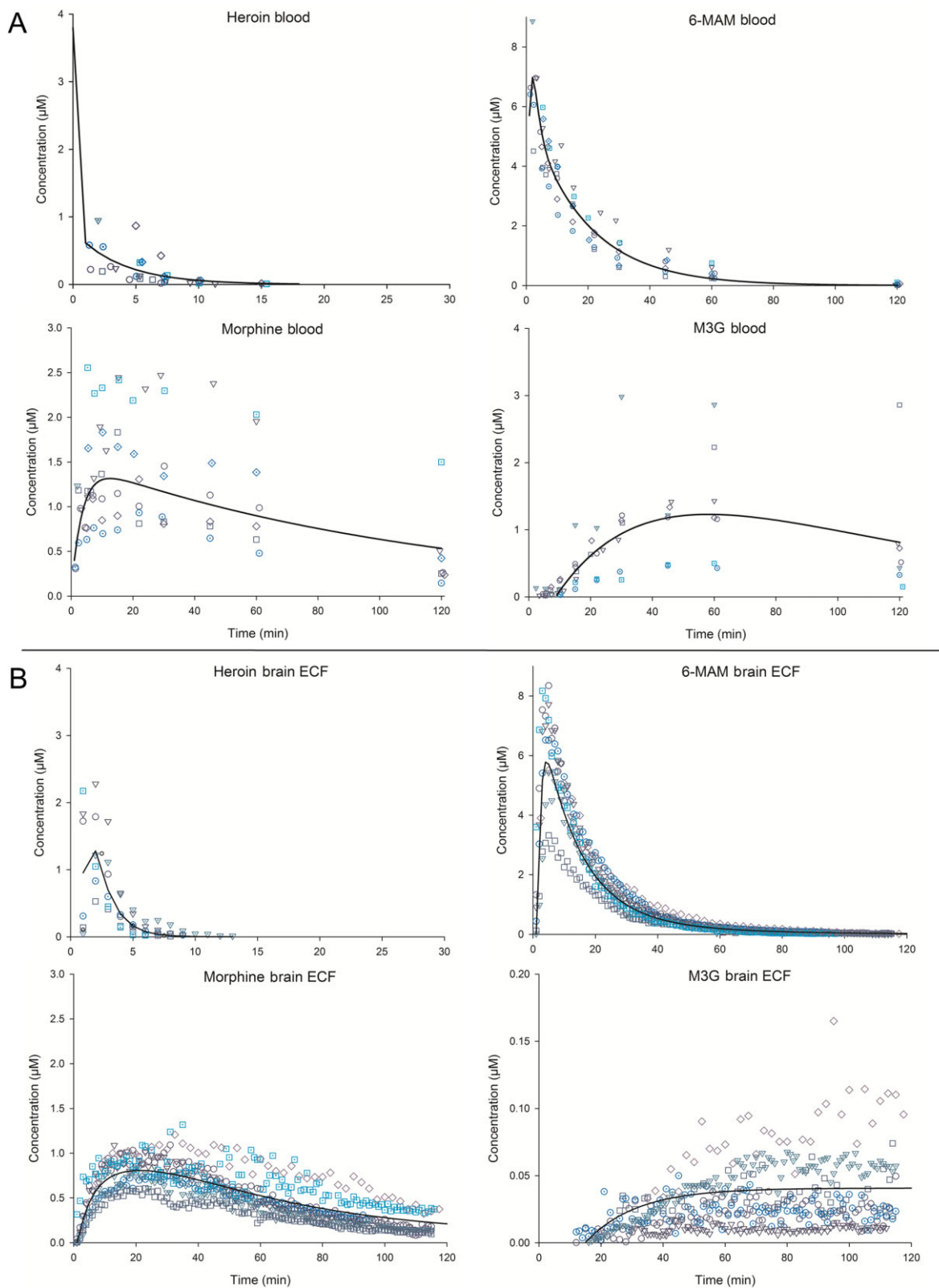
**Table 4**

Pharmacokinetic parameters after i.v. bolus injection. (All parameters are based on population-fitted data in Kinetica.)

Drug	Parameter	Blood	Brain ECF
Heroin	$AUC_{last}$ ( $\mu\text{M}\cdot\text{min}$ )	3.1	5.9
	$C_{max}$ ( $\mu\text{M}$ )	3.8*	1.5
	$T_{max}$ (min)	0.0	1.5
	$t_{1/2}$ $\alpha$ phase (min)	0.3	–
	$t_{1/2}$ elimination phase (min)	2.7	0.9
	Cl (mL·min)	979	–
	Vd (mL)	3787	–
6-MAM	$AUC_{last}$ ( $\mu\text{M}\cdot\text{min}$ )	112.3	102.7
	$C_{max}$ ( $\mu\text{M}$ )	7.0	5.8
	$T_{max}$ (min)	2.0	4.3
	$t_{1/2}$ $\alpha$ phase (min)	5.8	9.6
	$t_{1/2}$ elimination phase (min)	12.8	23.3
	Cl (mL·min)	27	–
	Vd (mL)	493	–
Morphine	$AUC_{last}$ ( $\mu\text{M}\cdot\text{min}$ )	107.6	59.6
	$C_{max}$ ( $\mu\text{M}$ )	1.3	0.8
	$T_{max}$ (min)	12.6	21.3
M3G	$AUC_{last}$ ( $\mu\text{M}\cdot\text{min}$ )	109.3	3.6
	$C_{max}$ ( $\mu\text{M}$ )	1.2	0.04
	$T_{max}$ (min)	58.2	120**

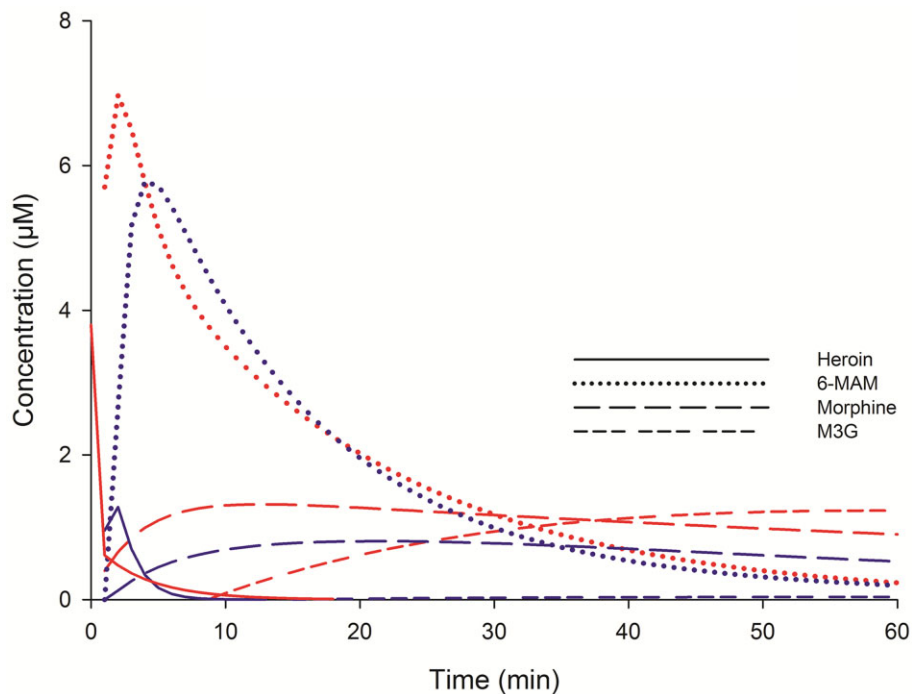
\* $C_{max}$  calculated in Kinetica must be considered as uncertain.

\*\* $T_{max}$  observed used for M3G in brain ECF.



**Figure 2**

Blood and brain ECF concentrations of heroin, 6-MAM, morphine and M3G after i.v. administration of  $3 \mu\text{mol}$  (1.3 mg) heroin. Points represent the observed concentrations (the different symbols representing the different animals), lines show the fitted values calculated in Kinetica software. (A) Observed and fitted values for each separate compound in blood ( $n = 8$ , M3G  $n = 7$ ). (B) Observed  $C_u$  and fitted values for each separate compound in brain ECF ( $n = 7$ , M3G  $n = 6$ ).



**Figure 3**

Blood and brain ECF concentrations of heroin, 6-MAM, morphine and M3G after i.v. administration of 3 µmol (1.3 mg) heroin. Lines show the fitted values calculated in Kinetica software. Red lines: fitted blood values time 0–60 min ( $n = 8$ , M3G  $n = 7$ ). Blue lines: fitted brain ECF values from time 0 to 60 min ( $n = 7$ , M3G  $n = 6$ ).

population-fitted results, 6-MAM reached blood concentrations about five times higher than morphine, with a  $C_{\max}$  at 7 µM and  $T_{\max}$  after 2 min. The decline in 6-MAM blood concentration curve was also biphasic, with an initial  $\alpha$  phase (between 3 and 5 min) with an apparent  $t_{1/2}$  of 5.8 min, and a terminal elimination phase (between 18 and 120 min) with a  $t_{1/2}$  of 12.8 min. In the  $\alpha$  phase, heroin was still present and could therefore influence the concentration curve for 6-MAM, whereas virtually all heroin was cleared from the blood during the terminal elimination phase. The morphine concentrations increased gradually, with a  $C_{\max}$  of 1.3 µM after 12.6 min ( $T_{\max}$ ). The blood level of M3G increased even slower than morphine, with a  $C_{\max}$  of 1.2 µM after 58 min ( $T_{\max}$ ). The Cl for heroin from blood was estimated to be 979 mL·min<sup>-1</sup>, and 27 mL·min<sup>-1</sup> for 6-MAM. Vd for heroin was calculated to be 3787 mL, and 493 mL for 6-MAM, by Kinetica.

### Brain ECF pharmacokinetics

The concentration versus time profiles are shown in Figure 2, panel B, and the calculated pharmacokinetic values are listed in Table 4. The calculated  $C_{\max}$  for heroin was 1.5 µM, with  $T_{\max}$  after 1.5 min. Heroin levels declined below LLOQ after about 10 min, with an apparent  $t_{1/2}$  of 0.9 min. 6-MAM reached concentrations approximately four and seven times higher than heroin and morphine, respectively, with a  $C_{\max}$  of 5.8 µM after 4.3 min ( $T_{\max}$ ). The decline in 6-MAM brain ECF concentration curve was biphasic, with an initial  $\alpha$  phase (between 6 and 26 min) that showed an apparent  $t_{1/2}$  of

9.6 min, and a following terminal phase (between 80 and 120 min) with a  $t_{1/2}$  of 23.3 min. Morphine levels increased during a longer period and reached  $C_{\max}$  of 0.8 µM after 21.3 min ( $T_{\max}$ ). The brain ECF levels of M3G increased even slower than morphine, with an apparent  $C_{\max}$  of 0.04 µM. The M3G level was still increasing towards the end of experiment, after 120 min.

### Relation between drug concentrations in blood and brain ECF

Figure 3 shows the fitted results for the concentrations of heroin and metabolites in blood and brain ECF. The concentration of heroin in brain ECF increased until reaching a maximum 1.5 min after the bolus injection, indicating that the absorption into this compartment lasted for at least this period of time. The concentration of 6-MAM in brain ECF reached a  $C_{\max}$  approximately 2.3 min after the  $T_{\max}$  for 6-MAM in blood. After  $C_{\max}$  was reached in brain ECF, the concentration of both heroin and 6-MAM declined in parallel with the respective concentrations in blood, with an initial time lag of a few minutes. The concentration of morphine and M3G in brain ECF reached  $C_{\max}$  approximately 10 min and more than 60 min after the respective  $T_{\max}$  values in the blood.

### Opioid concentrations in brain tissue

Due to technical problems with the sampling set-up, some rats ( $n = 5$ ) were killed during the experimental period of 120 min at 2, 35, 70, 71 and 80 min and their brains removed

immediately for measurement of drug content. Brains from other animals ( $n = 5$ ) killed at the end of the experimental period were also analysed. The concentration of heroin in brain tissue from the rat killed after 2 min was  $1.25 \text{ nmol}\cdot\text{g}^{-1}$ , from the rat killed after 35 min, the concentration was  $0.018 \text{ nmol}\cdot\text{g}^{-1}$ , whereas heroin levels below LLOQ were found in the brains collected at later time points. The concentrations of 6-MAM in the brain tissue were 18.36, 1.70, 0.16, 0.06 and  $0.07 \text{ nmol}\cdot\text{g}^{-1}$  for each of the time points indicated above respectively. In brains collected after 120 min, the mean concentration of 6-MAM was  $0.08 \text{ nmol}\cdot\text{g}^{-1} \pm 0.03 \text{ SEM}$  ( $n = 5$ ). The concentrations of morphine were 1.16, 1.33, 0.03, 0.77 and  $1.34 \text{ nmol}\cdot\text{g}^{-1}$  for each respective time point. In brains collected after 120 min the mean concentration was  $0.57 \text{ nmol}\cdot\text{g}^{-1} \pm 0.02 \text{ SEM}$  ( $n = 5$ ).

## Discussion

In the present study we investigated the distribution of heroin and its metabolites in blood and brain after an i.v. bolus injection of heroin to freely moving rats. When concentration measurements started, from 1 min onwards, the levels of heroin were relatively low in blood and brain ECF, and the levels of 6-MAM increased rapidly in both compartments and amply exceeded that of heroin already after 1 min in blood and after 2 min in brain ECF. The results showed that 6-MAM was the predominating compound present in blood and brain during the first 30 min after heroin administration. In brain ECF, the compartment where binding to opioid receptors involved in euphoria and reward takes place (Hammarlund-Udenaes, 2010; Pan and Pasternak, 2011), the maximum 6-MAM concentration was about four and seven times higher compared with heroin and morphine respectively. Moreover, the AUC for 6-MAM in the brain ECF was about twice as high as for morphine, and approximately 17 times higher compared with heroin. The AUC ratios between brain ECF and blood was 1.9, 0.9, 0.6 and 0.03 for heroin, 6-MAM, morphine and M3G, respectively, indicating that transfer of heroin and 6-MAM were mainly mediated by passive diffusion, whereas both morphine and M3G have a restricted transport over the BBB. However, the AUC ratio for heroin was probably deviating from its true value and was more likely closer to 1, as the extrapolation to  $C_{\text{max}}$  for heroin at time zero was difficult to perform due to the rapid metabolism and distribution. The true concentration at time zero was more likely around  $145 \mu\text{M}$ , calculated by dividing the administered dose with blood volume of  $6.9 \text{ mL}/100 \text{ g}$  rat (Probst *et al.*, 2006), resulting in an AUC in blood of about  $8 (\mu\text{M}\cdot\text{min})$  and a brain ECF : blood concentration ratio of about 0.7. Our present results were in line with previous findings from our laboratory showing that 6-MAM reached much higher  $C_{\text{max}}$  and AUCs than heroin and morphine after s.c. injections of heroin in mice (Andersen *et al.*, 2009), as measured in both whole blood and brain tissue samples. Thus, the high level of 6-MAM compared with heroin and morphine found shortly after heroin administration in the previous study (Andersen *et al.*, 2009) was not a consequence of the s.c. route of administration, as similar relationships between 6-MAM and heroin or morphine also were observed after i.v. bolus administration in the present study. In par-

ticular it should be noted that even though the dose used in this study was relatively high, there was no indication of saturation of the metabolic capacity in blood, as high concentration of 6-MAM was obtained rapidly.

MORs mediate the rewarding and reinforcing effects of heroin and morphine (Johnson and North, 1992) (for reviews see Koob and Bloom, 1988; Feltenstein and See, 2008). Brain striatum, which has a high abundance of  $\kappa$ -opioid receptors and MORs (Kling *et al.*, 2000), plays an important role regarding the effects seen after use of drugs of abuse (Everitt and Robbins, 2005). Further, in order to measure opiate concentrations in brain ECF for 1 min sampling periods and to get adequate recovery measurements, it was essential to use a 4 mm microdialysis membrane. The rat striatum allows the use of such long dialysis probe in a relative homogenous area. Thus, in this study, opiate concentrations were sampled from both ventral and dorsal striatum. 6-MAM binds to MORs with an affinity similar to morphine (Inturrisi *et al.*, 1983), but with a greater efficacy (Selley *et al.*, 2001). Because the binding to the MORs takes place on the extracellular surface of the neurons, our measurements of 6-MAM in striatal brain ECF indicated that 6-MAM should be important for the acute effects seen shortly after heroin intake, as is observed in the study of Andersen *et al.* (2009). The higher and faster increase of 6-MAM compared with morphine in brain ECF further substantiates the potentially important role of 6-MAM in mediating the acute effects seen after i.v. administration of heroin, as the rate of increase in concentration has been shown to be important for determining the potency of a drug of abuse (Volkow *et al.*, 2004; Samaha and Robinson, 2005). Whether this applies to all effects observed after heroin administration, for example, analgesia is however open for discussion. There are, for example, evidence for several MOR subtypes with selective binding affinities for opioid agonists present in varying concentrations throughout different brain areas (Bare *et al.*, 1994; Zimprich *et al.*, 1995; Pan *et al.*, 1999; 2000; 2001; 2009; Abbadie *et al.*, 2000; Xu *et al.*, 2009). These receptor subtypes mediate different downstream signal pathways, resulting in different analgesic and behaviour effects (Rossi *et al.*, 1995a,b; 1997; Pan *et al.*, 1999; Schuller *et al.*, 1999) (for review see Pasternak, 2010).

As can be observed in Figure 3, heroin entered the brain ECF quickly and attained a  $C_{\text{max}}$  already 1.5 min after the i.v. administration. At this point in time, the brain ECF 6-MAM concentration was already of the same order of magnitude and continued to rise thereafter, reaching the  $C_{\text{max}}$  4.3 min after the heroin injection when almost no heroin was found in the brain ECF. Thus, the brain ECF 6-MAM concentrations appeared to reflect the 6-MAM concentrations in the blood, both with respect to time course and concentrations, and not the brain ECF concentrations of heroin. The rats became rigorous and got respiratory depression immediately after heroin administration, when both heroin and 6-MAM levels were present in blood and brain ECF, but such observations were not systematically protocolled during the experiment, because this study focused mainly on pharmacokinetics. Even though morphine was present shortly after heroin injection, its levels in blood and brain were much lower than for heroin and 6-MAM in this initial stage, and the contribution of morphine to the observed effects was probably limited. This is supported by ongoing studies, where equimolar doses



of morphine do not result in rigor and only mild respiratory depression and sedation are observed.

Our results revealed a rapid elimination of heroin in blood, with an ultra-rapid distribution phase with a  $t_{1/2}$  of at least 0.3 min and a terminal elimination phase with  $t_{1/2}$  of 2.7 min. To our knowledge, there are no previous reports on *in vivo* heroin  $t_{1/2}$  in rats, but these findings are in accordance with earlier findings of  $t_{1/2}$  of 2.5 min in mice (Way *et al.*, 1960) and 1.3–7.8 min in humans (Rook *et al.*, 2006a). A traditional explanation for the fast disappearance of heroin from blood has been its putative fast transfer to the brain, in addition to its rapid hydrolysis (Oldendorf *et al.*, 1972; Umans and Inturrisi, 1982). The distribution of drugs from the blood stream across the BBB to the brain depends largely on their lipophilicity, the existence of active transport systems and the extent of protein binding. The partition coefficients in octanol/water have been calculated to be 0.85, 0.61, -0.07 and -1.12, for heroin, 6-MAM, morphine and M3G, respectively (Avdeef *et al.*, 1996), indicating a high lipophilicity for heroin and 6-MAM. It is well known that highly lipophilic drugs easily pass the BBB by passive diffusion and are redistributed to the brain (Oldendorf *et al.*, 1972; Umans and Inturrisi, 1981). Therefore, a fast transfer of heroin to the brain based on its lipophilicity could explain in part the low concentration of heroin in the blood, but would also imply that heroin should rapidly reach a relative high initial concentration in brain ECF. The  $C_{max}$  for heroin in brain ECF was however relatively low compared with 6-MAM, and the same appeared to AUC for heroin compared with AUC for 6-MAM. The relatively low concentration of heroin in brain ECF could however have been due to heroin distributing more rapidly than 6-MAM to other brain tissue compartments than ECF. It has been shown that drug concentrations measured in ECF may not correlate with the concentrations measured in total brain homogenate (Hammarlund-Udenaes, 2010). In the present study, heroin concentration in brain tissue from a rat killed after 2 min was of 1.29 nmol·g<sup>-1</sup> compared with 18.90 nmol·g<sup>-1</sup> for 6-MAM, indicating that heroin did not have a higher distribution to brain tissue than 6-MAM. A similar relationship between the concentrations of heroin and 6-MAM was also seen for the rat killed after 35 min. Further, *in vitro* studies in mice (Boix *et al.*, 2013) showed a much slower metabolism of heroin to 6-MAM in brain tissue compared with blood, observations that also corroborate with ongoing *in vitro* experiments in our laboratory in rats (to be published). We found a very large Vd for heroin compared with 6-MAM based on blood measurements. The present study did not investigate to which organs heroin distributed, but fat tissue might be a likely candidate.

In summary, the present study of i.v. bolus administration of heroin to rats indicated a rapid metabolism of heroin before it reached the brain, suggesting that 6-MAM was the main substance transported over the BBB shortly after i.v. injection of heroin. These results extended previous observations in our laboratory, in experiments where heroin was given s.c. (Andersen *et al.*, 2009; Boix *et al.*, 2013), showing that 6-MAM is the dominating compound in blood and brain also after rapid i.v. injection. We were further able to show that this also applied to 6-MAM concentrations in brain ECF, the relevant compartment where opioids interact with their receptors to attain their main effects. This rapid increase of

6-MAM concentration in the striatal brain area can explain the differences in relation to the acute effects and the drug addiction potential of heroin as compared with morphine. These findings are important as results from studies with morphine are frequently generalized to heroin. 6-MAM was the predominant substance present in brain ECF after heroin administration during the first 30 min and is likely to be responsible for the effects of heroin registered during this period. 6-MAM also reached concentrations several fold higher than those ever reached by morphine. This further emphasizes the importance of 6-MAM as the main metabolite of heroin rather than/in addition to morphine, and calls for including 6-MAM in future studies. Our results might also indicate new possibilities to manipulate acute heroin effects by interfering with the levels of the metabolite, 6-MAM, in blood, for example, by binding with antibodies, where some interesting studies have been performed the recent years (Stowe *et al.*, 2011; Pravetoni *et al.*, 2012; Schlosburg *et al.*, 2013) (for reviews see Anton *et al.*, 2009; Kinsey *et al.*, 2009).

## Acknowledgement

We would like to thank Bjørg S. Pettersen for her assistance in sample preparation and chemical analysis. This study has been partly supported by the grant 196621/V50 from the Norwegian Research Council.

## Conflict of interest

The authors state no conflict of interest.

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