Role of 6-monoacetylmorphine in the acute release of striatal dopamine induced by intravenous heroin

A. Gottås1, F. Boix1, E. L. Øiestad1, V. Vindenes1 and J. Mørland1,2

1 Department of Drug Abuse Research and Method Development, Division of Forensic Medicine and Drug Abuse Research, Norwegian Institute of Public Health, Pb. 4404, Nydalen, 0403 Oslo, Norway
2 Institute of Clinical Medicine, University of Oslo, Oslo, Norway

Abstract

After injection, heroin is rapidly metabolized to 6-monoacetylmorphine (6-MAM) and further to morphine. As morphine has been shown to increase striatal dopamine, whereas 6-MAM has not been studied in this respect, we gave i.v. injections of 3 μmol 6-MAM, morphine or heroin to rats. Opioids were measured in blood, and dopamine and opioids in microdialysate from brain striatal extracellular fluid (ECF), by UPLC-MS/MS. After 6-MAM injection, 6-MAM ECF concentrations increased rapidly, and reached Cmax of 4.4 μM after 8 min. After heroin injection, 6-MAM increased rapidly in blood and reached Cmax of 6.4 μM in ECF after 8 min, while ECF Cmax for heroin was 1.2 μM after 2 min. Tmax for morphine in ECF was 29 and 24 min following 6-MAM and heroin administration, respectively, with corresponding Cmax levels of 1 and 2 μM. Dopamine levels peaked after 8 and 14 min following 6-MAM and heroin administration, respectively. The dopamine responses were equal, indicating no dopamine release by heroin per se. Furthermore, 6-MAM, and not morphine, appeared to mediate the early dopamine response, whereas morphine administration, giving rise to morphine ECF concentrations similar to those observed shortly after 6-MAM injection, did not increase ECF dopamine. 6-MAM appeared accordingly to be the substance responsible for the early increase in dopamine observed after heroin injection. As 6-MAM was formed rapidly from heroin in blood, and was the major substance reaching the brain after heroin administration, this also indicates that factors influencing blood 6-MAM concentrations might change the behavioural effects of heroin.

Received 19 November 2013; Reviewed 14 December 2013; Revised 25 January 2014; Accepted 27 January 2014; First published online 27 February 2014

Key words: Drug abuse, dopamine, heroin, metabolite kinetics, microdialysis.

Abbreviations: AUCDA, AUC6-MAM, AUCMorphine, AUCHeroin; area under the concentration-time curve of dopamine, 6-MAM, morphine or heroin. Emax: maximum dopamine increase, defined as maximum effect. Cmax: maximum concentration (opioids). Tmax: time to reach Cmax or Emax. GABA: gamma-Aminobutyric acid. VTA: ventral tegmental area.

Introduction

Heroin is rapidly metabolized to 6-MAM, which is further metabolized to morphine (for overview see Rook et al., 2006a). This metabolism takes place in blood, liver, brain and other organs (Way et al., 1965; Lockridge et al., 1980). Heroin has much lower affinity (Inturrisi et al., 1983; Gianutsos et al., 1986) and intrinsic efficacy (Selley et al., 2001) to the μ-opioid receptors compared to its metabolites 6-MAM and morphine. Of the two active metabolites, 6-MAM is the dominating compound present during the first half hour after intake of heroin in blood, in humans (Rook et al., 2006b) as well as in rodents (Andersen et al., 2009; Gottås et al., 2013). This prevalence of 6-MAM can also be observed in the brain of rodents (Andersen et al., 2009), especially in brain extracellular fluid (ECF) (Gottås et al., 2013), the relevant compartment where opioids interact with their receptors to attain their main behavioural effects. Previous studies by our group have shown that the acute psycho-stimulating effects of heroin in mice are mainly mediated by 6-MAM and not morphine (Andersen et al., 2009).

An important effect of drugs of abuse, considered essential to their rewarding effects, as well as their ability to cause long-term neuroplastic changes (for reviews see Kauer and Malenka, 2007; Dacher and Nugent, 2011), is their ability to increase dopaminergic transmission in striatum (Church et al., 1987; Johnson and North, 1992) (for reviews see Nestler, 2005; Feltenstein and See, 2008). For opioids this is thought to occur mainly through the activation of μ-opioid receptors on GABA-interneurons.
with subsequent suppression of GABA inhibition of VTA dopaminergic cells (Johnson and North, 1992), leading to the release of dopamine in their target areas, e.g., the ventral (Di Chiara and Imperato, 1988a; Hemby et al., 1995; Wise et al., 1995) and dorsal striatum (Di Chiara and Imperato, 1988b). Whereas morphine has repeatedly been shown to increase striatal dopamine levels (Cadoni and Di Chiara, 1999; Vindenes et al., 2009), 6-MAM, has, as far as we know, not been studied with respect to its ability to provoke dopaminergic responses when given as such, or when formed as a consequence of heroin administration. In the present study we wanted to see if systemic administration of 6-MAM could increase striatal dopamine, and to what extent it is involved in the striatal dopamine overflow occurring shortly after acute heroin administration. To accomplish this goal, microdialysis was used to monitor simultaneously dopamine and EFC opioid concentrations from striatum, after i.v. administration of either 6-MAM, morphine or heroin.

Materials and methods

Animals and conditions

Male Spraque–Dawley rats from Harlan laboratories (The Netherlands) weighing about 250–300 g at arrival were used (n = 16). Each rat was housed in an individual cage (to avoid injury to the rat or the implants) in standard housing conditions (08:00–20:00 hours lights on), with free access to food and water. The experimental protocol was approved by the Norwegian Animal Research Authority and carried out in concordance to Norwegian regulation and international standards.

Experimental protocol

Surgical implantations of catheters and guide cannulas were performed by Harlan Laboratories (The Netherlands), with some modifications from previously established procedures in our laboratory (Gottås et al., 2012). In brief, the animals were implanted with two round-tip polyurethane catheters, one in the carotid artery and the other in the femoral vein. Two brain microdialysis guide cannulas (AT6.14.4, AgnTho’s, Lidingö, Sweden and CMA 12, CMA Microdialysis, Solna, Sweden) were implanted at the following coordinates relative to bregma: anterior (A): +0.5 mm; lateral (L): ±3.0 mm and lowered 4.0 mm ventrally into the striatum (Paxinos and Watson, 1998). The placement of the two different guide cannulas was laterally counterbalanced by switching their implant side on every other animal. The animals were shipped by car transport at least 3 d after surgery and left for acclimatization for at least 5 d after arrival. About 18 h before the experiment, the microdialysis probes (AT6.14.4, AgnTho’s, Lidingö, Sweden and CMA 12 Elite PAES, CMA Microdialysis, Solna, Sweden) were inserted into their respective guide cannulas. The metal free AT6.14.4 probe was used for sampling of the opioids (Gottås et al., 2012), whereas the standard CMA 12 was used for dopamine sampling. The microdialysis probes were perfused with Ringer’s solution at 0.2 μl min⁻¹ and the animal was left for acclimatization overnight.

The experiment was carried out as previously described (Gottås et al., 2012). Briefly, on the following day, the perfusion solution in the AT6.14.4 probe (opioid sampling) was switched to a Ringer’s solution containing deuterated recovery calibrators and the flow increased to 2 μl min⁻¹ flow for both probes. The sample collection intervals were 1 min for the opioids and 2 min for dopamine. Dopamine was sampled for about 30 min before injection of the drug to quantify the baseline level. The rat then received an i.v. bolus injection (0.1 ml) of 3 μmol (corresponding to approximately 10 μmol kg⁻¹) of either heroin (12.8 mg ml⁻¹), 6-MAM (12.5 mg ml⁻¹), or morphine (9.7 mg ml⁻¹) through the femoral vein catheter, followed by 0.3 ml of a physiological saline solution to ensure correct infusion of the injected substance. The dose chosen was high enough to quantify the low levels of heroin in brain ECF, both in the absorption and elimination phase (Gottas et al., 2012, 2013), without causing deleterious overdose side effects. Dialysate samples were collected for 120 min. Blood samples were taken from the carotid artery. During sampling collection, the animals were observed by one experimenter for evident behavioural effects. If such effects occurred, they were logged. After the experiment, the brain was immediately removed and frozen in liquid nitrogen (N₂). Probe location was later confirmed by histological analysis of brain slices, stained with methylene blue, obtained using a cryostat HM 550 (Microm International GmbH, Walldorf, Germany).

Chemical analysis

Sample preparation and analysis were performed according to established methods in our laboratory for analysis of opioids in dialysate (Gottås et al., 2012) and blood samples (Karinen et al., 2009; Gottås et al., 2013), and dopamine in dialysate samples (Gottås et al., in preparation, briefly presented in supplementary information).

Microdialysis probe recovery

The relative recoveries for each MD probe (AT6.14.4) and opioid was calculated by retrodialysis as described in (Gottås et al., 2012), and the values used for determining the concentration of unbound analyte (C_u) in brain ECF. The recovery values did not differ significantly from those published previously (Gottås et al., 2012, 2013).

Probe recovery was not determined for the dopamine microdialysis probes (CMA 12 Elite), as the objective
was to quantify the increase in dopamine concentration from baseline.

Chemicals and reagents
Chemicals and reagents used were as published previously (Gottås et al., 2012). Dopamine, used as analytical standard, was obtained from Sigma-Aldrich (Switzerland). Dopamine-d3, used as analytical internal standard, was obtained from Chiron (Norway).

Opioid stock and working solutions were prepared as in our previous publication (Gottås et al., 2012), and dopamine stock and working solutions as presented in supplementary information. The heroin solution for administration was freshly made at least once a week, and had a content of 6-MAM of no more than 1–3% prior to injection.

Data analysis
Due to occasional technical problems with sampling or analytical equipment, blood opioid data from one animal and dopamine data from two animals were unavailable. Dopamine data from one animal were, in addition, excluded due to incorrect location of the probe.

To compensate for blood sampling at differing times between the animals and incomplete data sets, phamacokinetic data curves were fitted to opioid blood and microdialysis data using the program Kinetica 5.1 (Thermo Fisher Scientific Inc., USA). All results were fitted by an extravascular model, except for blood data of heroin, 6-MAM, and morphine after their respective i.v. administration, which were fitted by an i.v. bolus model. The model with the lowest Akaike information criteria (AIC) was selected in order to assure the best fitting for each analyte (Ludden et al., 1994; Glatting et al., 2007).

Dopamine concentrations after drug administration were expressed as the difference between the value measured and the mean baseline value for the same animal. Concentrations of opioids and dopamine are expressed as mean±S.E.M. of all animals in each treatment group. Mean values from each treatment group were further used in a non-compartmental analysis (mixed log linear model implemented in the Kinetica software) to calculate the area under the concentration–time curve (AUC) from time zero to last sample time for dopamine and opioids in brain ECF and blood. The AUC was also calculated for 20 min periods corresponding to a standard sampling time often applied in microdialysis experiments. Additionally, the maximum dopamine increase, defined as maximum effect (E_max), maximum opioid concentration (C_max), and the time to reach both (T_max), were calculated. When several values were around E_max and the difference was not more than 0.1 nM, the first peak was chosen as E_max. Other blood and brain ECF opioid pharmacokinetic values are presented as supplementary material (Tables S1.1–1.6).

Statistical analysis
The effect of the drug treatments on dopamine was analysed statistically using a general linear model with ‘drug’ as between-subjects factor with three levels and ‘time’ as repeated within-subjects factor. For each drug, significant differences of each sampling point against baseline were analysed using the pairwise comparisons of the estimated means of a general linear model with ‘time’ as repeated within-subject factor. All the statistical analyses were performed using the SPSS 20 statistical package (IBM Corp., Armonk, New York, USA). A p<0.05 was considered statistically significant.

Results
Behavioural observations
Immediately after injection of heroin or 6-MAM, the animals showed evident signs of sedation through the duration of the experiment, with a period where myoclonus followed by increased muscle rigour and reduced respiration rate was observed. The myoclonus was most prominent after 6-MAM administration. Indication of cyanosis, with change in blood colour, was also observed at blood sampling, mostly during the first 5–10 min. Rats receiving morphine were less sedated, and woke up towards the end of the experiment; no signs of muscle rigidity or cyanosis were observed in these animals.

6-MAM injection
The concentration of 6-MAM in blood 1 min after 6-MAM injection (around the first sampling point) was 27.5±4.4 μM, with a subsequent biphasic decline (Supplementary material, Fig. S1-A). The blood morphine concentrations increased gradually, with a C_max of 1.6±0.2 μM after 22±7.8 min. 6-MAM rapidly entered brain ECF, reaching a C_max of 4.4±0.9 μM after 8±1 min, with a subsequent biphasic decline. Morphine levels in brain ECF slowly increased during a longer period, reaching a C_max of 1.0±0.2 μM after 29±4 min. Roughly, the concentration–time profiles of 6-MAM and morphine in ECF, after T_max was reached, followed those measured in blood. The total AUC of 6-MAM (AUC_{6-MAM}) in brain ECF was 131±27 μM*min, whereas the total AUC for morphine (AUC_{morphine}) was 79±13 μM*min (Fig. 1).

The dopamine concentrations in the brain ECF rapidly increased to an E_max of 9.7±4.7 nM, reached after 8±1 min (Fig. 1). The dopamine concentration declined rapidly for 5–10 min after E_max, before it stabilized slightly over baseline levels until a second minor increase occurred about 80 min after injection and lasting for about 30 min, with a maximum increase of 0.9±0.7 nM at approximately 100 min. The total AUC for dopamine (AUC_{DA}) was 91±16 nM*min. AUC_{DA} was 50 nM*min for the first 20 min after 6-MAM injection, when also...
the AUC$_{6\text{-MAM}}$ was highest (Fig. 1). During the subsequent 20 min periods both AUC$_{DA}$ and AUC$_{6\text{-MAM}}$ declined, while AUC$_{\text{Morphine}}$ increased. The small increase in AUC$_{DA}$ observed for the 20–30 min period after 80 min occurred when both AUC$_{6\text{-MAM}}$ and AUC$_{\text{Morphine}}$ were declining.

**Morphine injection**

To obtain more information on a possible role of morphine in the dopamine increase measured after 6-MAM injection, morphine was given to some animals. After morphine injections, the mean concentration of morphine in blood after 1 min (first sampling point) was $31.5\pm6.1\,\mu M$, with a subsequent biphasic decline (Supplementary material, Fig. S1-B). In brain ECF, the $C_{\text{max}}$ for morphine was $0.4\pm0.1\,\mu M$, reached after $6\pm1\,\text{min}$, with a subsequent biphasic decline (Fig. 2). The total ECF AUC$_{\text{Morphine}}$ was $14\pm2\,\mu M\cdot\text{min}$. The morphine brain ECF concentrations roughly followed the blood concentrations with regard to time profile and level, but were about a tenth of the levels observed in blood.

After morphine injection, the brain ECF dopamine concentrations slowly increased to an $E_{\text{max}}$ of $1.2\pm0.6\,\text{nM}$, reached after $46\pm22\,\text{min}$ (Fig. 2). Thereafter, the dopamine levels slowly declined towards baseline, but were still slightly higher than baseline at the end of the experiment. The total AUC$_{\text{DA}}$ was $66\pm23\,\text{nM}\cdot\text{min}$, and was highest in the period 40 to 80 min after morphine injection, when AUC$_{\text{Morphine}}$ was declining (Fig. 2).

**Heroin injection**

After heroin injection, the concentration of heroin in blood after 1 min (first sampling point) was $1.4\pm0.2\,\mu M$, with a subsequent biphasic decline, and concentrations below LLOQ already after 10–30 min (Supplementary material, Fig. S1-C). The corresponding concentration of 6-MAM in blood after 1 min was $19.9\pm2.9\,\mu M$, with a subsequent biphasic decline. The morphine blood concentrations increased gradually, with a $C_{\text{max}}$ of $1.7\pm0.2\,\mu M$ after $10\pm2\,\text{min}$. The brain ECF heroin concentrations roughly reflected those measured in blood with respect to time profile and level, and reached a $C_{\text{max}}$ of $1.2\pm0.4\,\mu M$ after $2\,\text{min}$, falling below LLOQ 10–33 min after the injection (Fig. 3). 6-MAM in brain ECF reached a $C_{\text{max}}$ of $6.4\pm1.0\,\mu M$ after $8\pm1\,\text{min}$. The decline in 6-MAM brain ECF concentration curve was biphasic, with an apparent $t_{1/2}$ of $10.7\pm0.7\,\text{min}$ in $\alpha$-phase and $22.0\pm2.1\,\text{min}$ during the terminal phase. Brain ECF morphine levels increased during a longer period, reaching a $C_{\text{max}}$ of $2.0\pm0.5\,\mu M$ after $24\pm2\,\text{min}$. The total AUC of heroin (AUC$_{\text{Heroin}}$) in brain ECF was $5\pm1\,\mu M\cdot\text{min}$, whereas the total AUC$_{6\text{-MAM}}$ in brain ECF was $137\pm18\,\mu M\cdot\text{min}$ and total AUC$_{\text{Morphine}}$ was $115\pm32\,\mu M\cdot\text{min}$ (Fig. 3).

After heroin administration, the brain ECF dopamine concentration rapidly increased to an $E_{\text{max}}$ of $4.7\pm1.7\,\text{nM}$ reached after $14\pm1\,\text{min}$ (Fig. 3). The dopamine concentration declined gradually after $E_{\text{max}}$ falling towards, but not reaching, baseline levels throughout the experimental period, and several minor short-term increases.
were observed. The total AUC<sub>DA</sub> was 197±23 nM*min, and was highest during the first 20 min period, when also the AUC<sub>Heroin</sub> and AUC<sub>6-MAM</sub> were highest (Fig. 3). The AUC<sub>DA</sub> then declined for two subsequent 20 min periods, as did AUC<sub>Heroin</sub> and AUC<sub>6-MAM</sub>. Thereafter, AUC<sub>DA</sub> increased slightly for the period 60–80 min, when both AUC<sub>6-MAM</sub> and AUC<sub>Morphine</sub> were still declining.

Pairwise comparison of the dopamine responses following heroin and 6-MAM injection indicated no difference for the first 20 min (p=0.74), the period where 6-MAM concentration was higher than morphine. A significant difference was, however, observed for the rest of the experiment (p<0.05).

Controls

Three animals received an i.v. injection of physiological saline solution (0.4 ml) prior to the start of the experimental protocol presented above. This administration of saline did not affect the brain ECF dopamine levels during the following hour (Supplementary material, Fig. S2).

There was no major difference in probe location among the treatment groups (Supplementary material, Fig. S3). It should be noted that the mean baseline dopamine level in the heroin treated rats was markedly higher than for the 6-MAM and morphine treated rats (4.3±0.8, 0.8±0.4 and 0.9±0.3 nM, respectively). We found no obvious reason for this from probe locations. However, there were overlapping baseline levels in the 6-MAM and heroin groups, as animals with high baseline levels (approximately 2 nM) were found in both groups. The magnitude of the dopamine response after heroin and 6-MAM injection was, however, not related to the respective baseline levels.

Discussion

In the present study we primarily investigated the relationship between the ECF pharmacokinetics of 6-MAM, morphine and heroin when given i.v. and the striatal dopamine response in an in vivo rat model. The i.v. administration of 6-MAM and heroin gave a rapid increase of the extracellular levels of dopamine in striatum, whereas morphine induced a slow rise.

Access of heroin metabolites to brain ECF

After i.v. injection of 6-MAM, it rapidly crossed the blood–brain barrier (BBB) and reached ECF concentrations quite similar to those measured in blood, with a corresponding concentration vs. time profile in ECF roughly similar to that in blood (Supplementary material, Fig. S1-A). These observations were similar to previous measurements in mouse brain after s.c. 6-MAM administration (Andersen et al., 2009). Morphine crossed the BBB to a much lower extent (Supplementary material, Fig. S1-B), as also shown in previous studies (Aasmundstad et al., 1995; Letrent, 1999; Tunblad et al., 2003). After heroin injection, the concentration of 6-MAM rapidly reached concentrations several times higher than heroin, both in blood and brain ECF.

<table>
<thead>
<tr>
<th>Compound</th>
<th>AUC&lt;sub&gt;0–20 min&lt;/sub&gt;</th>
<th>AUC&lt;sub&gt;20–40 min&lt;/sub&gt;</th>
<th>AUC&lt;sub&gt;40–60 min&lt;/sub&gt;</th>
<th>AUC&lt;sub&gt;60–80 min&lt;/sub&gt;</th>
<th>AUC&lt;sub&gt;80–100 min&lt;/sub&gt;</th>
<th>AUC&lt;sub&gt;100–120 min&lt;/sub&gt;</th>
<th>AUC&lt;sub&gt;0–120 min&lt;/sub&gt;</th>
<th>Tmax</th>
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<tr>
<td>Brain ECF [dopamine]</td>
<td>4</td>
<td>13</td>
<td>19</td>
<td>14</td>
<td>10</td>
<td>6</td>
<td>66</td>
<td>46</td>
</tr>
<tr>
<td>Brain ECF [morphine]</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>14</td>
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Fig. 2. Mean brain ECF concentrations opiates (n=3) and mean (±S.E.M.) changes in dopamine ECF levels (n=3) after i.v. administration of 3 μmol (1.0 mg) morphine. AUC levels in 20 min intervals are presented for each compound along with AUC<sub>total</sub> and Tmax.
(Supplementary material, Fig. S1-C), in accordance with recent findings by our group (Gottås et al., 2013); the 6-MAM and morphine concentrations in brain ECF were further similar after heroin injection and injection of an equimolar dose of 6-MAM.

**Effect of 6-MAM on striatal dopamine release**

In order to evaluate 6-MAM’s role in heroin induced increase in dopamine release, the effect of systemically injected 6-MAM was first studied. Dopamine peaked 8 min after i.v. 6-MAM injection, which closely corresponded with the time of \( C_{\text{max}} \) for 6-MAM in ECF. At this point, morphine ECF concentration was still increasing, reaching a much lower \( C_{\text{max}} \) about 21 min later. From inspection of the ECF concentration–time curves for 6-MAM, morphine and dopamine, the early dopamine increase mirrored the increase of 6-MAM, which also reached much higher concentrations than morphine at this time (Fig. 1). The closer relation between \( T_{\text{max}} \) for 6-MAM and dopamine, and the lag between dopamine and morphine strongly indicated that 6-MAM, and not morphine, mediated the early effect on dopamine after 6-MAM injection. A possible additional effect by morphine could occur. The mechanisms underlying the second minor increase in dopamine seen about 80 min after injection of 6-MAM, when both the concentrations of 6-MAM and morphine were declining, are unclear, but could be related to morphine in brain ECF, as the concentration of morphine at that time had surpassed that of 6-MAM.

**Effect of morphine on striatal dopamine release**

After i.v. administration of morphine, dopamine in ECF peaked after 46 min, long after \( T_{\text{max}} \) for morphine in ECF. This indicated that morphine had a delayed effect, as the dopamine increase was not closely related to \( T_{\text{max}} \) morphine in ECF. Such a delay has previously been observed after i.v. morphine injection (Pontieri et al., 1995), and after direct injection of morphine into the VTA (Leone et al., 1991), which might suggest possible slow receptor kinetics (Pleuvry, 2005) for morphine. When we compared the morphine concentrations in ECF during the first 10 min period after injection of morphine with those after injection of 6-MAM, their AUC\(_{\text{morphine}} \) were quite similar (Fig. 4). While we found a marked dopamine increase after 6-MAM administration during this period, it was negligible after morphine injection, which indicated that morphine did not contribute to the early dopamine increase observed after 6-MAM injection. It could be argued that the concentration–time
higher, however not significant, after heroin than after 6-MAM injection. If heroin by itself had stimulated dopamine release, we would have assumed a higher AUC$_{DA}$ after heroin injection due to the combined/additive effects with 6-MAM and not, as observed, an almost equal dopamine response the first 20 min ($p=0.74$). We therefore concluded that the early dopamine response after i.v. heroin injection was due to 6-MAM present in the ECF after heroin conversion to 6-MAM, mostly occurring in blood (Boix et al., 2013; Gottås et al., 2013). Following the first 20 min after the i.v bolus injection of heroin, the dopamine response differed from the dopamine response detected after i.v. 6-MAM, and was probably caused by the interaction between the different heroin metabolites. As the AUC ratios between 6-MAM and morphine, as well as the $T_{max}$ for morphine, were different after heroin and 6-MAM injection (Figs 1 and 3), it cannot be excluded that the more rapid increase of morphine in ECF after heroin injection would mediate the observed difference in dopamine response after the first 20 min. Assuming a similar affinity of 6-MAM and morphine for the $\mu$-opioid receptor (Inturrisi et al., 1983; Gianutsos et al., 1986), despite a higher intrinsic efficacy of 6-MAM (Selley et al., 2001), the competitive binding to receptors between morphine and 6-MAM would likely influence and modulate the dopamine response depending on the concentration ratio in ECF. However, higher dopamine baseline levels were observed before heroin administration, a factor which could have contributed to the observed results.

It has previously been shown that the rapid uptake of drugs of abuse, e.g. cocaine, into the brain can affect their reinforcing effects, and is associated with getting 'high' (Balster and Schuster, 1973; Volkow et al., 1995). In addition to the rapid uptake of a drug, it has been emphasized that not only the increase in dopamine concentration per se, but also the rate of the increase are important for the experience of euphoria in humans (Volkow et al., 2004, 2007). The rapid and marked increase in brain ECF concentration of 6-MAM, and not morphine, was closely related to the pronounced dopamine response observed early after i.v. heroin administration in the present study. Thus, 6-MAM is likely important, and possibly also the main factor, for the highly rewarding effect of heroin. Accordingly, 6-MAM has probably a more central role in the reinforcing and addictive effects observed for heroin than previously acknowledged.

To conclude, we showed that an i.v. injection of 6-MAM resulted in a rapid increase of dopamine release in striatum, and that this increase was consistent with an increase of 6-MAM in brain ECF. Further, we demonstrated that 6-MAM formed from heroin was the main compound responsible for the early increase in striatal dopamine seen after i.v. heroin injection. This study thus demonstrated the importance of 6-MAM in mediating the early dopamine release observed after heroin.
injection, moving the focus from morphine to the initial heroin metabolite, 6-MAM, in this respect. As the initial dopamine increase after exposure to a drug of abuse is considered important for its acute (Volkow et al., 2004; Samaha and Robinson, 2005), as well as long term behavioural effects (for reviews see Volkow et al., 2007; Dacher and Nugent, 2011), it would be interesting to see future studies aiming at interfering with 6-MAM, which is mostly formed from heroin before it reaches the brain (Boix et al., 2013; Gottås et al., 2013), and how this could subsequently influence heroin induced behaviour.

Supplementary material

For supplementary material accompanying this paper, visit http://dx.doi.org/10.1017/S1461145714000169

Acknowledgments

This study has been partly supported by the grant 196621/V50 from the Norwegian Research Council. We thank Åse Ripel for important insight and valuable discussions to the analytical methods, and Bjørg Pettersen, Elisabeth Nerem and Gerd Wenche Brochmann for their exceptional technical assistance.

Statement of Interest

None.

References