ELSEVIER

Pharmacology, Biochemistry and Behavior 73 (2002) 883–892

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

# Pharmacokinetic differences of morphine and morphine-glucuronides are reflected in locomotor activity

Marte Handal[a,*], Merete Grung[a,1], Svetlana Skurtveit[a,b], Åse Ripel[a], Jørg Mørland[a]

[a]*National Institute of Forensic Toxicology, P.O. Box 495, Sentrum, N-0105 Oslo, Norway*
[b]*Norwegian Institute of Public Health, P.O. Box 4404, Nydalen, N-0403 Oslo, Norway*

Received 5 March 2002; received in revised form 19 June 2002; accepted 21 June 2002

**Abstract**

The main metabolites of morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G), have been considered to participate in some of the effects of morphine. There is limited knowledge of the pharmacokinetics and dynamics of morphine and the main metabolites in mice, but mice are widely used to study both the analgesic effects and the psychomotor effects of morphine. The present study aimed to explore pharmacokinetic differences between morphine and morphine-glucuronides in mice after different routes of administration, and to investigate how possible differences were reflected in locomotor activity, a measure of psychostimulant properties. Mice were given morphine, M3G or M6G by different routes of administration. Serum concentrations versus time curves, pharmacokinetic parameters and locomotor activity were determined. Intraperitoneal administration of morphine reduced the bioavailability compared to intravenous and subcutaneous administration, but not so for morphine-glucuronides. The two morphine-glucuronides had similar pharmacokinetics, but morphine demonstrated higher volume of distribution and clearance than morphine-glucuronides. The present results demonstrated no locomotor effect of M3G, but a serum concentration effect relationship for morphine and M6G. When serum concentrations and effect changes were followed over time, there was some right hand shifts with respect to locomotor activity, especially during the declining phase of the concentration curve and particularly for M6G.


*Keywords:* Morphine; Morphine-3-glucuronide; Morphine-6-glucuronide; Pharmacokinetics; Locomotor activity; Mice

## 1. Introduction

Morphine causes a spectrum of effects ranging from analgesia and respiratory depression to central nervous stimulation and euphoria. The mechanisms by which morphine produces its effects are not clear. In recent years, considerable attention has been paid to the potential role of morphine metabolites in the elicitation of pharmacological responses. Morphine-6-glucuronide (M6G) causes analgesia in animals (Gong et al., 1991; Paul et al., 1989) and has been found to have antinociceptive effects in humans (Hanna et al., 1990; Osborne et al., 1988). Conversely, morphine-3-glucuronide (M3G) is inactive in man (Penson et al., 2000) and has in some studies been reported to possibly antagonize the effects of morphine and M6G in rats (Gong et al., 1992; Smith et al., 1990). Contradictory results are reported concerning this possible functional antagonism (Hewett et al., 1993; Lipkowski et al., 1994; Suzuki et al., 1993).

In humans, morphine is metabolized to M3G and M6G. These glucuronides by far exceed the plasma concentration of morphine shortly after as well single dose administrations as during chronic treatment (Sawe et al., 1985). However, we and others have reported that mice and rats produce no or only trace amounts of M6G (Grung et al., 1998; Milne et al., 1996). In, e.g., mice, the effects of morphine can be studied undisturbed by M6G formation, which could otherwise add its pharmacodynamic effects to the effects of morphine.

The limbic and striatal dopamine (DA) neurons are implicated in the locomotor stimulant effects of morphine and other opioids in rats (Joyce and Iversen, 1979; Kalivas and Duffy, 1987; Kalivas and Stewart, 1991), although it is recognized that part of the opioid-induced motor stimulation

* Corresponding author. Tel.: +47-23-40-78-00; fax: +47-22-38-32-33.
*E-mail address*: marte.handal@labmed.uio.no (M. Handal).
[1] Present address: Norwegian Institute for Water Research, P.O. Box 173, Kjelsås, N-0411 Oslo, Norway.

0091-3057/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0091-3057(02)00925-5

is DA-independent (Cornish et al., 2001; Kalivas et al., 1983). The dopaminergic system, specifically the dopaminergic mesocortical and mesolimbic systems, have been shown to play a major role in reward (Shippenberg and Elmer, 1998; Spanagel and Weiss, 1999; Wise and Bozarth, 1982) and, accordingly, locomotor activity can yield information on the rewarding and reinforcing effects of drugs.

The pharmacokinetics of a drug is important both in the design and interpretation of pharmacodynamic studies. The pharmacokinetics of morphine and its metabolites in mice have not been fully described although mice are widely used in pharmacodynamic studies to elucidate the effects of morphine. Accordingly, only few studies (Pacifici et al., 1994, 2000) have addressed some of the pharmacokinetic–pharmacodynamic relations in mice.

We have earlier (Grung et al., 1998, 2000; Mørland et al., 1994) demonstrated that M6G can cause locomotor activation similar to morphine in mice. The present experiments were designed to assess the pharmacokinetic differences between morphine and the morphine-glucuronides in mice with special emphasis on consequences of different routes of administration and, subsequently, to explore if possible pharmacokinetic differences were reflected as differences in rewarding and reinforcing properties of morphine and M6G measured by locomotor activity.

## 2. Materials and methods

### 2.1. Animals

C57BL/6J-Bom adult (7–8 weeks old), drug-naive, male mice (14–24 g body weight at testing) from Bomholt, Denmark were used for the experiments. The animals were housed eight per cage in the animal vivaria at the Norwegian Institute of Public Health, Oslo, Norway, at room temperature of 22 ± 1 °C. The animals were kept on a 12/12-h light/dark schedule with light period from 07:00 to 19:00 h. The mice were housed in the vivaria for at least 5 days prior to experiments. They had free access to food and water throughout the acclimatization period. They were fasted overnight before the experiments. Each animal was tested once. The experimental protocol of this study was approved by the Norwegian Review Committee for the use of Animal Subjects.

### 2.2. Materials

Morphine hydrochloride (mol. wt. 375.9) was purchased from Norsk Medisinaldepot (Oslo, Norway), morphine-6-β-D-glucuronide dihydrate (mol. wt. 497.5) from Ultrafine Chemicals (Manchester, England), morphine-3-β-D-glucuronide (mol. wt. 461.5) from Sigma (St. Louis, USA). The drugs were dissolved in 0.9% saline. Acetonitrile from Labscan (Dublin, Ireland) was HPLC-grade. All other reagents were analytical grade.

### 2.3. Treatment

#### 2.3.1. Pharmacokinetic studies

Each animal was given one bolus injection (80 μmol/kg) of morphine, M3G or M6G. The three drugs were administrated by three different routes; one group of animals received the drug as intravenous injections, one as subcutaneous injections and one as intraperitoneal injections. The intravenous injections were given in total volumes of 0.1 ml/10 g mouse, the subcutaneous injections in total volumes of 0.05 ml/10 g mouse and the intraperitoneal injections in total volumes of 0.2 ml/10 g mouse.

#### 2.3.2. Locomotor studies

Two groups with 12 animals in each group were injected with morphine, one group with 40 μmol/kg and one with 80 μmol/kg. Another two groups with the same number of animals were injected with the same doses of M6G. In each of the four treatment group, half the animals ($n = 6$) received the drug by intraperitoneal administration and the other half by a subcutaneous injection. In addition, four animals received M3G (40 μmol/kg) and another four saline, respectively (two animals by the intraperitoneal route and two by the subcutaneous route). The intraperitoneal injections were given in total volumes of 0.2 ml/10 g mouse and the subcutaneous injections in total volumes of 0.1 ml/10 g mouse.

### 2.4. Blood sampling and sample purification

In the pharmacokinetic studies, two to seven animals were killed at each time point by heart blood sampling under $CO_2$–anesthesia. After 60 min at room temperature, the blood was centrifuged for 10 min at $1670 \times g$ and serum was stored at −18 °C until analyses were preformed.

### 2.5. HPLC analysis

Morphine, M3G and M6G in serum were analyzed by HPLC with ultraviolet and electrochemical detection employing automated solid phase extraction described by Svensson (1986) and Svensson et al. (1982) and modified by Aasmundstad et al. (1993) with 50 μl serum. The limit of detection was 0.5 μM for morphine, 0.4 μM for M3G and 0.2 μM for M6G, and the interassay variability was < 10% for all components.

### 2.6. Pharmacokinetic calculations

Mean serum concentrations were calculated at each time point. The serum drug half-lives, total clearance (CL) and volume of distribution ($V_D$) were calculated from the mean serum concentrations obtained after intravenous administration. These data were calculated using a noncompartmental pharmacokinetic data analysis by PK Solutions 2.0 software (Summit Resarch Services, Ashland, OH, USA).

The data analysis of the three drugs were carried out with the two terms option (absorption/distribution and elimination) and the elimination phases were determined from 30 to 60 min to the last concentration depending on what gave the best curve fit. When M3G was formed as a metabolite of morphine the three terms option (absorption, absorption/distribution and elimination) was used with elimination phases as above. Following intravenous administration, the software calculated an initial concentration (not shown in figures). The areas under the serum concentration versus time curves (AUC) were calculated based on mean serum concentrations. To indicate the distribution of the data, a range of AUC was calculated. A maximum AUC was calculated by using the mean concentration values + S.E.M. concentration at each time point. Similarly, the minimum AUC was calculated using the mean concentration values − S.E.M. concentration values at each time point.

### 2.7. Locomotor activity

Each animal was tested individually in an activity chamber of a Digiscan optical animal activity monitoring system (Omnitec Electonics, Columbus, USA). The chamber size was 20 × 20 cm with infrared beam spacing of 2.5 cm. Each animal was individually habituated in an activity chamber for 90 min before injections. After habituation, the mouse was gently removed from the activity chamber and injected with morphine or M6G in another room. Immediately following injection, it was gently returned to the same activity chamber. Locomotor activity was measured for a time period of 600 min following morphine and M6G injections, respectively. Each animal's score was expressed as activity counts per 5-min period. A battery of different activities was measured as an expression of locomotor activity (Grung et al., 1998). Based upon our previous study, we have focused on one activity, the total distance traveled, to present our results.

### 2.8. Data analysis

Statistical analyses were performed with SPSS version 10.0 statistical software. Data are presented as mean ± S.E.M. unless otherwise stated. Locomotor activity data were compared by Student's $t$ test. $P$ values of less than .05 were taken as statistically significant.

## 3. Results

### 3.1. Pharmacokinetic studies

#### 3.1.1. Administration of morphine

Fig. 1 shows the serum concentration versus time curves of morphine and its metabolite M3G in mice receiving a single dose of 80 μmol/kg morphine as intravenous, intraperitoneal or subcutaneous injections. The AUCs are given in Table 1. The AUC for morphine following intraperitoneal administration was only 47% of that following intravenous administration. After subcutaneous administration, however, the AUC was of the same magnitude as following intravenous administration. The serum concentration of morphine following the intraperitoneal injection reached a maximum concentration ($c_{max}$) of approximately 12 μM after 10 min. Following subcutaneous administration, a $c_{max}$ of approximately 18 μM was reached after 25 min. The initial concentration following intravenous administration (calculated by PK solution software) was approximately 60 μM (not shown in figure). There was no detectable amount of morphine at 180 min after administration regardless of administration route.

M3G was the main metabolite formed following morphine administration. Fig. 1 shows that detectable quantities of the glucuronide were found in plasma 5 min after morphine administration independent of administration route. Following intraperitoneal administration, the M3G concentration was substantially higher than the concentra-

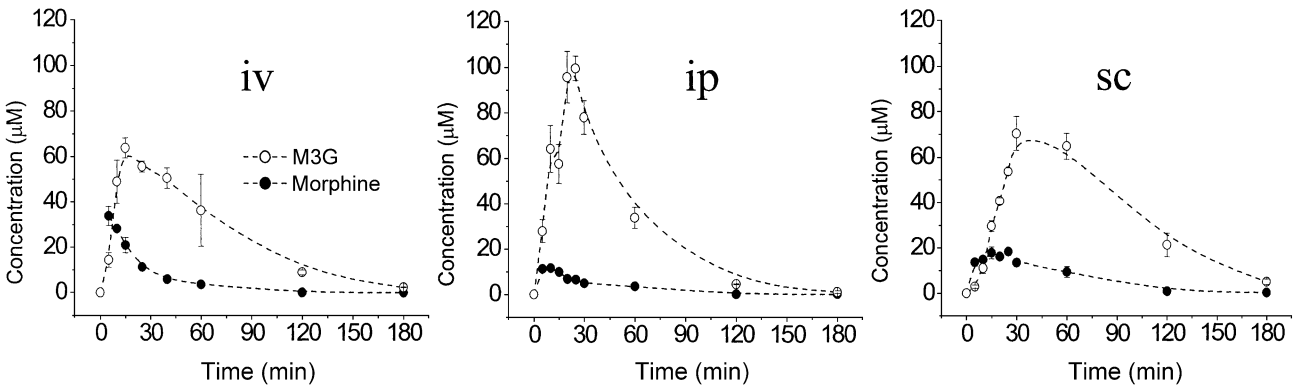


Fig. 1. Serum concentration versus time curves of morphine and its metabolite M3G following single dose administration of 80 μmol/kg morphine by intravenous (iv), intraperitoneal (ip) and subcutaneous (sc) routes. Mean concentrations ± S.E.M. of groups of two to seven mice are given at each time point. When S.E.M. bars are not visible, they are smaller than the plot symbol.

Table 1
AUC of the serum concentration versus time curves of 80 μmol/kg morphine and its metabolite M3G following three different routes of single dose morphine administration[a]

| Route of administration | $AUC_{mean}$ ($AUC_{min} - AUC_{max}$)[b] | | |
|---|---|---|---|
| | Morphine (mmol·min·l$^{-1}$) | M3G (mmol·min·l$^{-1}$) | Ratio[c] M3G/morphine |
| Intravenous | 0.99 (0.88–1.10) | 4.53 (3.61–5.45) | 4.6 |
| Subcutaneous | 1.10 (0.88–1.32) | 6.47 (5.63–7.32) | 5.9 |
| Intraperitoneal | 0.47 (0.42–0.53) | 4.94 (4.35–5.54) | 10.5 |

[a] Numbers of analyzed blood samples at each time point were two to seven.
[b] AUC denotes area under the serum concentration versus time curves. $AUC_{mean}$ values were calculated as the AUC of the mean serum concentrations. The range is shown in brackets ($AUC_{min} - AUC_{max}$). $AUC_{min}$ was calculated as the area of the curve made up of the mean concentrations − S.E.M. at each time point and the $AUC_{max}$ was calculated as the area of the curve made up of the mean concentrations + S.E.M. at each time point.
[c] Ratios are based on mean AUC values.

Table 2
AUC of the serum concentration versus time curves of single dose administration of 80 μmol/kg M3G and M6G given by three different routes of administration[a]

| Route of administration | $AUC_{mean}$ ($AUC_{min} - AUC_{max}$)[b] | |
|---|---|---|
| | M3G (mmol·min·l$^{-1}$) | M6G (mmol·min·l$^{-1}$) |
| Intravenous | 3.35 (3.02–3.69) | 5.31 (4.71–5.89) |
| Subcutaneous | 5.21 (4.71–5.70) | 4.40 (3.88–4.91) |
| Intraperitoneal | 4.76 (4.32–5.20) | 3.81 (3.36–4.26) |

[a] Numbers of analyzed blood samples at each time point were two to five.
[b] Abbreviations and explanations as in Table 1.

tion of morphine already after 5 min. Table 1 shows the AUCs of the serum concentration versus time curves of M3G formed as a metabolite of morphine after the different routes of administration. The AUC ratio of M3G to morphine was about 10 following intraperitoneal administration compared to about 5–6 after the two other administration routes of morphine (Table 1). The serum concentration of M3G following intraperitoneal morphine administration reached a $c_{max}$ of approximately 100 μM after 25 min. Following the subcutaneous dose, a $c_{max}$ of about 70 μM was reached after 30 min and, following the intravenous administration, a $c_{max}$ of approximately 65 μM was reached after 15 min. There were detectable concentrations of M3G present during the entire experiment, however, less than 3 μM at 180 min.

No M6G was detected following the administration of morphine by any route.

### 3.1.2. Administration of morphine-glucuronides

The left panel of Fig. 2 shows the serum concentration versus time curves following a single dose of 80 μmol/kg M3G as intravenous, intraperitoneal and subcutaneous injections. There was no reduction in AUC when M3G was administered by intraperitoneal or subcutaneous administration compared to intravenous administration (Table 2). In fact, the AUC of the concentration versus time curve following intravenous administration was somewhat smaller than following the two other routes of administration. The maximal serum concentrations of M3G after intraperitoneal and subcutaneous doses were similar (around 120 μM), while the initial concentration following intravenous administration (calculated by PK solution software) was 180 μmol/l (not shown in figure). These concentrations were higher compared to the M3G concentrations obtained as a metabolite following injection of equimolar doses of morphine given intravenously or subcutaneously, but similar when compared to M3G concentrations after morphine intraperitoneal (compare Fig. 1 and left panel of Fig. 2). M3G was not detected at 180 min following subcutaneous administration and only very low concentrations ($\leq 3$ μM) were detected at the end of the experiment following the two other routes of administration. No M6G or morphine was detected at any time after the administration of M3G by any route.

The serum concentration versus time curves following M6G administration by intravenous, intraperitoneal and subcutaneous injections are shown in the right panel of Fig. 2. No marked differences in AUCs following subcutaneous and intraperitoneal administration routes were calcu-

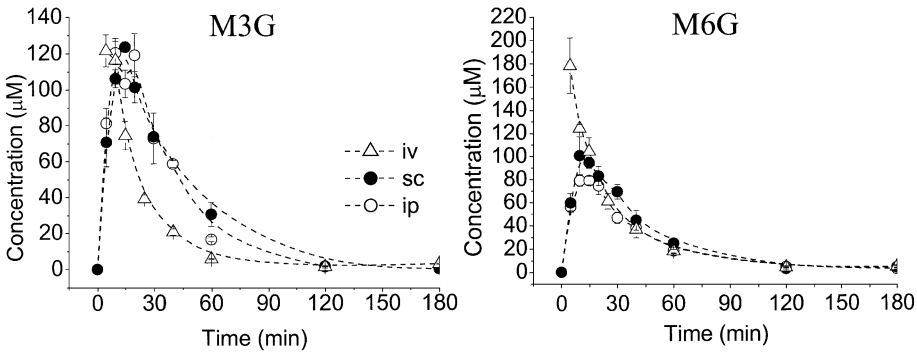


Fig. 2. Serum concentration versus time curves of single dose administration of 80 μmol/kg M3G and M6G, respectively. The drugs were administered by intravenous (iv), intraperitoneal (ip) and subcutaneous (sc) routes. Mean concentrations ± S.E.M. of groups of two to four (M3G) and two to six (M6G) mice are given at each time point, respectively. When S.E.M. bars are not visible, they are smaller than the plot symbol.

Table 3
Pharmacokinetic data[a]

| | Half-life (min) | $V_D$[b](l/kg) | CL[c] (ml/min/kg) |
|---|---|---|---|
| Morphine | 28 | 3.2 | 80 |
| M3G | 27 | 0.9 | 24 |
| M6G | 25 | 0.5 | 15 |

[a] The pharmacokinetic data are calculated based on mean concentrations following intravenous administration.
[b] $V_D$ denotes volume of distribution.
[c] Denotes total clearance.

lated (Table 2), but both areas tended to be lower than the AUC following intravenous administration. The serum concentration of M6G reached a $c_{max}$ of approximately 80 μM 10–15 min following intraperitoneal injection, while the $c_{max}$ following subcutaneous administration was slightly higher and approximately 100 μM. The initial concentration following intravenous administration (calculated by PK solution software) was approximately 260 μM (not shown in figure). We found detectable concentrations of M6G during the entire experiment, although lower than 4.0 μM at 180 min. No morphine or M3G were detected at any time point after the administration of M6G, by any route.

### 3.2. Pharmacokinetic data

The calculated pharmacokinetic data are presented in Table 3. The half-lives of the three drugs determined from mean serum concentrations were similar and within the range of 25–28 min, while marked differences were observed for $V_D$ and CL. $V_D$ and CL of both glucuronides were similar. Morphine had 3.5–6.5 times higher $V_D$ and CL values than the glucuronides.

### 3.3. Locomotor activity experiments

The locomotor activity expressed as mean total distance traveled per 5 min, following administration of 40 and 80

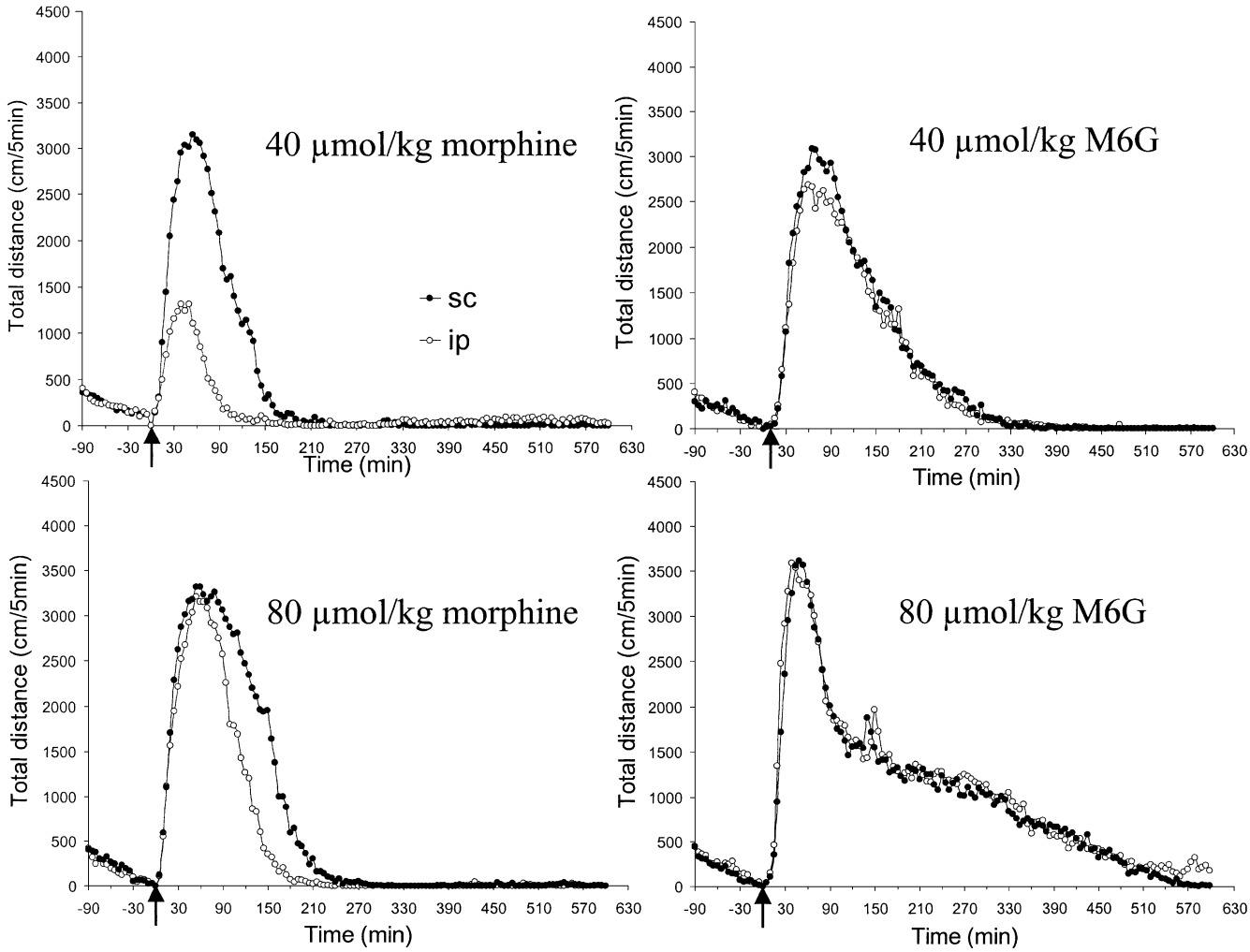


Fig. 3. Locomotor activity following morphine (left panels) and M6G (right panels) administration by intraperitoneal (ip) and subcutaneous (sc) routes presented as mean total distance traveled per 5 min at each 5-min interval. The arrows indicate the time of drug administration following 90 min of habituation. The time scales are given in relation to the moment of injection. In all groups, $n = 6$. S.E.M. bars were omitted for clarity.

Table 4
Data from locomotor activity curves (total distance traveled) after different doses and administration routes of morphine and M6G[a]

| | Morphine | | | | M6G | | | |
|---|---|---|---|---|---|---|---|---|
| | 40 μmol/kg | | 80 μmol/kg | | 40 μmol/kg | | 80 μmol/kg | |
| | Intraperitoneal | Subcutaneous | Intraperitoneal | Subcutaneous | Intraperitoneal | Subcutaneous | Intraperitoneal | Subcutaneous |
| $AUC_{la}$[b] (m) | 187 ± 20 | 558 ± 64 * | 587 ± 72 | 857 ± 28 * * | 717 ± 78 | 784 ± 116 | 1290 ± 100 | 1215 ± 109[†] |
| $E_{max}$[c] (m/5 min) | 15 ± 2 | 33 ± 3 * | 35 ± 4 | 39 ± 1 | 30 ± 3 | 32 ± 4 | 37 ± 3 | 38 ± 3 |
| $t_{max}$[d] (min) | 42 ± 4 | 60 ± 4 * * | 65 ± 6 | 60 ± 8 | 77 ± 11 | 73 ± 4 | 42 ± 3 | 52 ± 4 |

[a] Data presented as mean ± S.E.M., $n = 6$ in all groups.
[b] $AUC_{la}$ denotes area under the locomotor activity curve.
[c] $E_{max}$ denotes maximum locomotor effect.
[d] $t_{max}$ denotes the time to reach maximum effect. Other abbreviations as in Table 1.
* Significant difference from intraperitoneal administration of the same dose of morphine ($P < .001$ by Student's $t$ test).
** Significant difference from intraperitoneal administration of the same dose of morphine ($P < .01$ by Student's $t$ test).
[†] Significant difference from the same dose of subcutaneous administration of morphine ($P < .05$ by Student's $t$ test).

μmol/kg morphine and M6G by the subcutaneous and intraperitoneal routes of administration are presented in Fig. 3. Corresponding mean values of the area under the activity curve ($AUC_{la}$), maximum locomotor effect ($E_{max}$) and the time to reach maximum effect ($t_{max}$) of the curves are presented in Table 4.

The experiments revealed that following ip administration of 40 μmol/kg morphine, the $E_{max}$ as well as the $AUC_{la}$ were significantly smaller and approximately 45% and 34% of the corresponding curve following subcutaneous administration, respectively (upper left panel in Fig. 3 and Table 4). Following the higher dose of morphine (80μmol/kg), the $AUC_{la}$, but not the $E_{max}$, was smaller (approximately 70%) than corresponding values following subcutaneous administration (lower left panel in Fig. 3 and Table 4). When M6G was administered, the $E_{max}$ and the $AUC_{la}$ of the curves following the two different routes of administration were equal both for animals receiving 40 and 80 μmol/kg (right panels Fig. 3 and Table 4). Further, there were no significant differences in $E_{max}$ or $AUC_{la}$ following subcutaneous administration of 40 μmol/kg morphine compared to the same dose of M6G. However, when the doses were 80 μmol/kg, the $AUC_{la}$ following M6G administration was larger then following morphine administration, while the corresponding $E_{max}$ were similar. M3G and saline did not induce increased locomotor activity in C57B6J mice (data not shown).

The time to reach maximum effect ($t_{max}$) was significantly shorter in the group receiving 40 μmol/kg morphine intraperitoneally compared to the mice receiving the same dose by subcutaneous administration (Table 4). In mice receiving either the high dose of morphine or M6G (both doses), there were no significant differences in $t_{max}$ between the two different administration routes of the same dose. When comparing the groups receiving subcutaneous administration of either morphine (both doses) or M6G (both doses), there were not any significant differences in $t_{max}$ (ranging from 52 to 73 min).

From the locomotor activity curves in Fig. 3, one can read that subcutaneous morphine administration induced locomotor activity increases that lasted for around 240 and 270 min following the dose of 40 and 80 μmol/kg, respectively. M6G administration induced locomotor activity increases that lasted longer, almost 360 min and at least for 570 min following 40 and 80 μmol/kg, respectively.

Other locomotor activities than total distance traveled showed the same pattern of activity changes as the total distance traveled shown in Fig. 3 (data not shown).

Fig. 4 shows hysteresis plots of the association between locomotor activity and serum concentrations of morphine and M6G, respectively. Both plots were counter clockwise plots and show that there was a delay in achieving locomotor activity response compared to the raise in serum concentrations for both drugs, but most pronounced for M6G. The maximum activity reached was in the same order of magnitude and was reached at approximately the same time for the two drugs. When the drug concentrations declined to zero, the locomotor activity was still present for a period of time, again most pronounced for M6G.

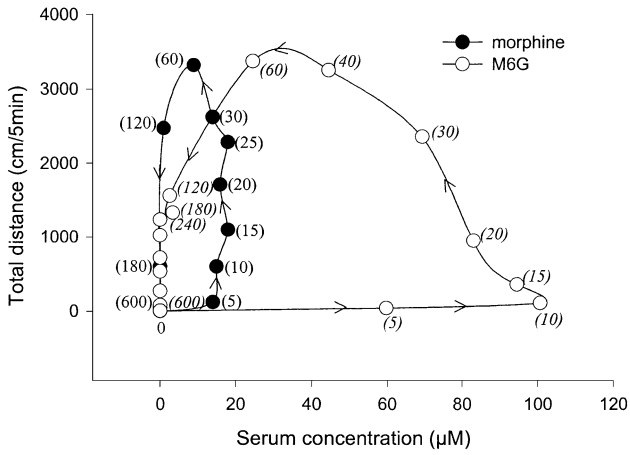


Fig. 4. Counter clockwise hysteresis plots of locomotor activity versus serum concentrations of morphine and M6G. Results following subcutaneous administration of 80 μmol/kg morphine or M6G, respectively, are given, $n = 6$ in each group. Numbers in brackets at plot symbols denotes the time of the actual measurement (M6G time points shown in italics) and arrows show the time series of the measurements.

## 4. Discussion

We found that following intraperitoneal administration of morphine the bioavailability was nearly 50% compared to intravenous and subcutaneous administration, and that intraperitoneal administration was followed by higher concentrations of the metabolite M3G. M6G was never detected after morphine administration. When M3G was given by the three different routes, there were no marked serum concentration difference, which was also the case when M6G was given intraperitoneally or subcutaneously. The half-lives for all three drugs were similar, but morphine had $V_D$ and CL values approximately 3.5–6.5 times higher than both glucuronides.

These results indicated that morphine given intraperitoneally is subject to substantial first pass metabolism in the liver giving rise to high concentrations of M3G, which were also found to rise earlier following intraperitoneal injections of morphine compared to the other two routes of administration. We are not aware of previous studies on this first-pass metabolism of morphine given intraperitoneally to mice. Studies in other species also seemed to be lacking. However, it has been demonstrated that oral administration morphine was followed by substantial first-pass metabolism in several species (Milne et al., 1996). Since the peritoneal cavity is drained mainly by the portal circulation (Benet et al., 1996), it was not surprising that the intraperitoneal route of administration would lead to first-pass metabolism comparable to that observed after oral administration.

The route of administration did not reduce the bioavailability of M3G when this substance was injected intraperitoneally, indicating that there was no hepatic first-pass metabolism of M3G. This seemed reasonable since the elimination of morphine-glucuronides has been shown almost exclusively to take place by renal secretion of unchanged drug (Milne et al., 1996). This was also in accordance with the findings in a study from our laboratory with isolated guinea pig and rat hepatocytes demonstrating that morphine-glucuronides were stable end products (Aasmundstad et al., 1993). There was a tendency that the AUC following intraperitoneal administration of M6G was reduced compared to intravenous, but not subcutaneous administration. A possible minor first-pass metabolism in the liver at the three-position of the molecule could explain this reduction and, accordingly, the small distinction between M3G and M6G.

We did not detect M6G following morphine administration. This was in agreement with results from other groups (Oguri et al., 1990; Pacifici et al., 1995; Zuccaro et al., 1997), and also in accordance with the low UDPGT activity towards the 6-hydroxyl group of the morphine molecule found in liver microsomes from mice (Kuo et al., 1991). Morphine was not detected following the administration of M3G or M6G. This indicated that there was no enterohepatic circulation similar to what has been shown in dogs (Garrett and Jackson, 1979). Our results concerning the differences between $V_D$ and CL for morphine on one hand and M3G and M6G on the other were in accordance with previous observations for other animal species and man (Milne et al., 1996). The low $V_D$ of the glucuronides were in accordance with the lower fat solubility of morphine-glucuronides compared to morphine and explained the high serum concentrations obtained for the glucuronides compared to morphine.

The second part of our study addressed whether the pharmacokinetic differences observed had importance to the pharmacodynamic responses that could be observed as locomotor activities. We found that the route of administration (subcutaneous and intraperitoneal) influenced the locomotor activity following morphine, but not M6G administration. Following intraperitoneal administration of 40 μmol/kg morphine, both the $AUC_{la}$ and $E_{max}$ were less than 50% of the locomotor activity after subcutaneous administration. Following the higher dose of morphine, the reduction in $AUC_{la}$ was not as pronounced as after the low dose and there was no difference in $E_{max}$. There were no significant differences in $AUC_{la}$ and $E_{max}$ of the locomotor activity curves between intraperitoneal and subcutaneous M6G for both doses tested, i.e., 40 and 80 μmol/kg. When the locomotor effects of subcutaneous morphine and M6G were compared, we found larger effects after M6G. The difference was statistically significant for the 80 μmol/kg dose measured as $AUC_{la}$ and was mainly due to a longer duration of the increased locomotor activity following M6G administration. M3G did not increase locomotor activity different from saline.

There was a close relationship between the pharmacokinetics of morphine with respect to bioavailability and the locomotor activity. The lower bioavailability of 80 μmol/kg morphine (48%) when administered intraperitoneally was reflected in reduced locomotor activity (approximately 30%) following ip compared to subcutaneous administration of the same dose of morphine in the pharmacodynamic study. The $E_{max}$ of the locomotor activity curves after the high dose of morphine were equal following the two routes of administration even though the $c_{max}$ was about 30% lower following intraperitoneal compared to subcutaneous administration. We have earlier showed that the locomotor activity following 80 μmol/kg morphine intraperitoneal gave rise to a maximum effect in dose response studies (Grung et al., 1998). Because of the possibility of a ceiling effect we also measured the locomotor activity after 40 μmol/kg morphine by intraperitoneal and subcutaneous administration. We found again a marked decrease of $AUC_{la}$ (66%) after intraperitoneal compared to subcutaneous administration and at this dose also the $E_{max}$ after intraperitoneal was reduced compared to subcutaneous administration (55%). The pharmacodynamic consequence of the pharmacokinetic differences between intraperitoneal and subcutaneous administration thus became clearer when the dose was lowered, demasking a ceiling effect.

For M6G the lack of difference in bioavailability between intraperitoneal and subcutaneous routes of administration of 80 μmol/kg, M6G was reflected in equal activities with respect to locomotor activity measured as $AUC_{la}$ and $E_{max}$. Reducing the dose to 40 μmol/kg did not reveal any concealed pharmacodynamic differences because of possible ceiling effects. To our knowledge, there are no previous studies on the locomotor activity of M6G comparing different routes of administrations.

Our data indicated that on dose basis, morphine and M6G were equipotent with respect to giving rise to the same $E_{max}$ following subcutaneous administration of both doses of the two substances. However, in serum, the concentrations following subcutaneous administration of the two drugs were very different. The $c_{max}$ following M6G administration was approximately 5.5 times that of morphine administration. This may indicate that M6G passes the blood–brain barrier (BBB) to a lesser degree than morphine. This is in agreement with previous studies that in different ways have studied the passage through the BBB (Bickel et al., 1996; Mignat et al., 1995; Aasmundstad et al., 1995). In rats, it was shown by microdialysis that the concentration difference between morphine and M6G in the extracellular fluid of the brain (ECF) was much smaller than the corresponding difference in plasma (Aasmundstad et al., 1995). If this also applies to mice, morphine and M6G concentrations in the ECF in our study would have been approximately equal. Since M6G has been shown to have comparable affinities for the μ receptor (Frances et al., 1992; Pasternak et al., 1987; Paul et al., 1989), this may explain the equal potencies of the two substances. This similarity in potency following systemic administration was in agreement with another study where place preference was studied (Abbott and Franklin, 1991), and with a study where analgesic effects of morphine and M6G were compared (Frances et al., 1990).

If one focuses at administration by the subcutaneous route, to avoid the disturbance of low bioavailability following intraperitoneal administration, the locomotor activity of both 80 μmol/kg morphine and M6G rose rapidly as the serum concentrations rose. However, there was a time delay, depicted by the counter clockwise hysteresis plot between the two curves (Fig. 4). The most probable explanation for this was that it takes time to distribute the drugs to their effect compartment, the brain, due to the crossing of the BBB. The time delay between the $c_{max}$ and $E_{max}$ to reach the maximum locomotor activity was in the same order of magnitude (approximately 35–40 min) following 80 μmol/kg morphine and M6G. This was in agreement with the study by Aasmundstad et al., in rats, which showed that M6G reached the peak ECF concentration at the same time as morphine (Aasmundstad et al., 1995). The peak concentration of both morphine and M6G in cerebral ECF was equally delayed (40 min) compared to the maximum concentration in serum. This was in agreement with our finding that $t_{max}$ of the locomotor activity was equal following morphine and M6G. The delay between the $c_{max}$ in serum and $E_{max}$ of the locomotor activity in our study was also of the same order of magnitude (35–40 min) as shown in the aforementioned study.

In morphine-treated mice the locomotor activity past $E_{max}$ fell relatively parallel to the serum concentration, but with an even greater time delay than between the maximum concentrations and effects. When the serum concentration reached zero, the locomotor activity was low and approached zero in about 90 min. One explanation for this time delay might be that the half-life of morphine in ECF was greater than in serum. This was in agreement with previous studies in rats reporting $t_{1/2}$ from 30 to 32 and 44 to 48 min in serum and ECF, respectively (Bouw et al., 2000; Aasmundstad et al., 1995). The time delay could also be explained in a pharmacodynamic manner; the binding of morphine to its receptor initiated intracellular processes that had a duration that was longer than the presence of morphine in ECF. Bouw et al. (2000) have estimated that 85% of the delay in antinociceptive effect of morphine in rats could be explained by the transport of morphine across the BBB. Their results also indicated a possible involvement of pharmacodynamic factors in explaining some of the delay.

In the first time period after passing $E_{max}$, the locomotor activity of M6G fell approximately parallel with the serum concentrations but with some delay. However, when the serum concentrations approached zero at 180 min, the locomotor activity was still high and only slowly descending, not even reaching zero at the end of the experiment 600 min after the drug injection. Clearly, the shape of the locomotor activity curve differed from what was seen with morphine. This indicated that M6G might have a longer half-life in ECF than morphine in agreement with what had been found in rats (Aasmundstad et al., 1995). The binding of M6G to another receptor than morphine; a specific M6G receptor or a splice variant of the μ receptor (Brown et al., 1997; Pasternak, 2001) might also explain or at least contribute to the difference. A difference between the locomotor activity curve shapes in the descending part of the curve was previously also shown in mice though not as pronounced as in our study (Uchihashi et al., 1996). The doses used by Uchihashi et al. were, however, much lower than those we tested. The longer duration of M6G effects compared to morphine had also been shown in mice when studying analgesic effects (Paul et al., 1989).

The limbic and striatal DA neurons are implicated in the locomotor stimulant effects of morphine and other opioids in rats (Joyce and Iversen, 1979; Kalivas and Duffy, 1987; Kalivas and Stewart, 1991). The relatively close temporary relationship between morphine serum concentrations and locomotor activity suggested that there was a close relationship between morphine concentrations in serum and ECF. This also indicated that the DA concentration or the DA effect paralleled the morphine concentrations in ECF relatively closely, in accordance with close temporal connections between ventral striatum DA-release and locomotor

activation in this strain of mice (Murphy et al., 2001). This probably also applies for M6G although the temporary relationship might be somewhat different especially in the late phase of the locomotor activity.

In the pharmacokinetic part of the study, we had only given very high doses of the three substances to assure us that they would be easy to detect in serum. The dose was so high that one could argue that circulatory effects could arise that would affect pharmacokinetic parameters. However, we had earlier administered up to 120 μmol/kg morphine and M6G without the animals behaving any different than following smaller doses (Grung et al., 1998). In pharmacokinetic studies, it is desirable to examine the fate of the studied substance in one subject. The amount of serum needed for the HPLC-analysis did not allow us to take serial blood samples of each mouse. Consequently, we had to use mean serum concentrations from several animals per time point to draw the concentration time curve, which increased the uncertainty in the data. For the purpose of this study, however, in comparing morphine with the glucuronides, we found that the uncertainty was acceptable. Addition of urine samples would have made the study more comprehensive if one wanted to fully explore the detailed pharmacokinetics of the drugs. However, this was not the aim of this study.

In conclusion, the behavioral parameter locomotor activity was closely related to serum concentrations of morphine following morphine administration both with respect to response and time course. The same was to some extent the case for M6G, although there was a considerable discrepancy in the later time periods with respect to an increased ratio between locomotor activity and M6G concentration. This might indicate important differences in intracerebral pharmacokinetics and/or dynamics with respect to locomotor activity between morphine and M6G.

## Acknowledgements

The authors thank Dr. T. Aasmundstad, Rikshospitalet, Oslo, Norway for fruitful discussions. This research was supported by grant no. 128575/330 from the division of Medicine and Health of The Research Council of Norway where M.H. is a research fellow.

## References

Aasmundstad TA, Ripel A, Bodd E, Bjørneboe A, Mørland J. Different biotransformation of morphine in isolated liver cells from guinea pig and rat. Biochem Pharmacol 1993;46:961–8.

Aasmundstad TA, Mørland J, Paulsen RE. Distribution of morphine-6-glucuronide and morphine across the blood–brain barrier in awake, freely moving rats investigated by in vivo microdialysis sampling. J Pharmacol Exp Ther 1995;275:435–41.

Abbott FV, Franklin KB. Morphine-6-glucuronide contributes to rewarding effects of opiates. Life Sci 1991;48:1157–63.

Benet LZ, Kroetz DL, Sheiner LB. Pharmacokinetics: the dynamics of drug absorption, distribution, and elimination. In: Hardman JG, Limbbird LE, Molinoff PB, Ruddon RW, Gilman AG, editors. Goodman and Gilman's the pharmacological basis of therapeutics. USA: McGraw-Hill, 1996. p. 3–27.

Bickel U, Schumacher OP, Kang YS, Voigt K. Poor permeability of morphine-3-glucuronide and morphine-6-glucuronide through the blood–brain barrier in the rat. J Pharmacol Exp Ther 1996;278:107–13.

Bouw MR, Gardmark M, Hammarlund-Udenaes M. Pharmacokinetic–pharmacodynamic modelling of morphine transport across the blood–brain barrier as a cause of the antinociceptive effect delay in rats—a microdialysis study. Pharm Res 2000;17:1220–7.

Brown GP, Yang K, Ouerfelli O, Standifer KM, Byrd D, Pasternak GW. $^{3}$H-Morphine-6beta-glucuronide binding in brain membranes and an MOR-1-transfected cell line. J Pharmacol Exp Ther 1997;282:1291–7.

Cornish JL, Nakamura M, Kalivas PW. Dopamine-independent locomotion following blockade of N-methyl-D-aspartate receptors in the ventral tegmental area. J Pharmacol Exp Ther 2001;298:226–33.

Frances B, Gout R, Campistron G, Panconi E, Cros J. Morphine-6-glucuronide is more mu-selective and potent in analgesic tests than morphine. Prog Clin Biol Res 1990;328:477–80.

Frances B, Gout R, Monsarrat B, Cros J, Zajac JM. Further evidence that morphine-6 beta-glucuronide is a more potent opioid agonist than morphine. J Pharmacol Exp Ther 1992;262:25–31.

Garrett ER, Jackson AJ. Pharmacokinetics of morphine and its surrogates: III. Morphine and morphine-3-monoglucuronide pharmacokinetics in the dog as a function of dose. J Pharm Sci 1979;68:753–71.

Gong QL, Hedner T, Hedner J, Bjorkman R, Nordberg G. Antinociceptive and ventilatory effects of the morphine metabolites: morphine-6-glucuronide and morphine-3-glucuronide. Eur J Pharmacol 1991;193:47–56.

Gong QL, Hedner J, Bjorkman R, Hedner T. Morphine-3-glucuronide may functionally antagonize morphine-6-glucuronide induced antinociception and ventilatory depression in the rat. Pain 1992;48:249–55.

Grung M, Skurtveit S, Aasmundstad TA, Handal M, Alkana RL, Mørland J. Morphine-6-glucuronide-induced locomotor stimulation in mice: role of opioid receptors. Pharmacol Toxicol 1998;82:3–10.

Grung M, Skurtveit S, Ripel A, Mørland J. Lack of cross-tolerance between morphine and morphine-6-glucuronide as revealed by locomotor activity. Pharmacol, Biochem Behav 2000;66:205–10.

Hanna MH, Peat SJ, Woodham M, Knibb A, Fung C. Analgesic efficacy and CSF pharmacokinetics of intrathecal morphine-6-glucuronide: comparison with morphine. Br J Anaesth 1990;64:547–50.

Hewett K, Dickenson AH, McQuay HJ. Lack of effect of morphine-3-glucuronide on the spinal antinociceptive actions of morphine in the rat: an electrophysiological study. Pain 1993;53:59–63.

Joyce EM, Iversen SD. The effect of morphine applied locally to mesencephalic dopamine cell bodies on spontaneous motor activity in the rat. Neurosci Lett 1979;14:207–12.

Kalivas PW, Duffy P. Sensitization to repeated morphine injection in the rat: possible involvement of A10 dopamine neurons. J Pharmacol Exp Ther 1987;241:204–12.

Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res Brain Res Rev 1991;16:223–44.

Kalivas PW, Widerlov E, Stanley D, Breese G, Prange AJ. Enkephalin action on the mesolimbic system: a dopamine-dependent and a dopamine-independent increase in locomotor activity. J Pharmacol Exp Ther 1983;227:229–37.

Kuo CK, Hanioka N, Hoshikawa Y, Oguri K, Yoshimura H. Species difference of site-selective glucuronidation of morphine. J Pharmacobiodyn 1991;14:187–93.

Lipkowski AW, Carr DB, Langlade A, Osgood PF, Szyfelbein SK. Morphine-3-glucuronide: silent regulator of morphine actions. Life Sci 1994;55:149–54.

Mignat C, Jansen R, Ziegler A. Plasma and cerebrospinal fluid concentrations of morphine and morphine glucuronides in rabbits receiving single and repeated doses of morphine. J Pharm Pharmacol 1995;47:171–5.

Milne RW, Nation RL, Somogyi AA. The disposition of morphine and its

3- and 6-glucuronide metabolites in humans and animals, and the importance of the metabolites to the pharmacological effects of morphine. Drug Metab Rev 1996;28:345–472.

Mørland J, Jones BL, Palomares ML, Alkana RL. Morphine-6-glucuronide: a potent stimulator of locomotor activity in mice. Life Sci 1994;55: PL163–8.

Murphy NP, Lam HA, Maidment NT. A comparison of morphine-induced locomotor activity and mesolimbic dopamine release in C57BL6, 129Sv and DBA2 mice. J Neurochem 2001;79:626–35.

Oguri K, Hanioka N, Yoshimura H. Species differences in metabolism of codeine: urinary excretion of codeine glucuronide, morphine-3-glucuronide and morphine-6-glucuronide in mice, rats, guinea pigs and rabbits. Xenobiotica 1990;20:683–8.

Osborne R, Joel S, Trew D, Slevin M. Analgesic activity of morphine-6-glucuronide. Lancet 1988;1:828.

Pacifici R, Patrini G, Venier I, Parolaro D, Zuccaro P, Gori E. Effect of morphine and methadone acute treatment on immunological activity in mice: pharmacokinetic and pharmacodynamic correlates. J Pharmacol Exp Ther 1994;269:1112–6.

Pacifici R, Pichini S, Altieri I, Caronna A, Passa AR, Zuccaro P. High-performance liquid chromatographic–electrospray mass spectrometric determination of morphine and its 3- and 6-glucuronides: application to pharmacokinetic studies. J Chromatogr, B, Biomed Appl 1995;664: 329–34.

Pacifici R, di Carlo S, Bacosi A, Pichini S, Zuccaro P. Pharmacokinetics and cytokine production in heroin and morphine-treated mice. Int J Immunopharmacol 2000;22:603–14.

Pasternak GW. Insights into mu opioid pharmacology the role of mu opioid receptor subtypes. Life Sci 2001;68:2213–9.

Pasternak GW, Bodnar RJ, Clark JA, Inturrisi CE. Morphine-6-glucuronide, a potent mu agonist. Life Sci 1987;41:2845–9.

Paul D, Standifer KM, Inturrisi CE, Pasternak GW. Pharmacological characterization of morphine-6 beta-glucuronide, a very potent morphine metabolite. J Pharmacol Exp Ther 1989;251:477–83.

Penson RT, Joel SP, Bakhshi K, Clark SJ, Langford RM, Slevin ML. Randomized placebo-controlled trial of the activity of the morphine glucuronides. Clin Pharmacol Ther 2000;68:667–76.

Sawe J, Kager L, Svensson Eng JO, Rane A. Oral morphine in cancer patients: in vivo kinetics and in vitro hepatic glucuronidation. Br J Clin Pharmacol 1985;19:495–501.

Shippenberg TS, Elmer GI. The neurobiology of opiate reinforcement. Crit Rev Neurobiol 1998;12:267–303.

Smith MT, Watt JA, Cramond T. Morphine-3-glucuronide—a potent antagonist of morphine analgesia. Life Sci 1990;47:579–85.

Spanagel R, Weiss F. The dopamine hypothesis of reward: past and current status. Trends Neurosci 1999;22:521–7.

Suzuki N, Kalso E, Rosenberg PH. Intrathecal morphine-3-glucuronide does not antagonize spinal antinociception by morphine or morphine-6-glucuronide in rats. Eur J Pharmacol 1993;249:247–50.

Svensson JO. Determination of morphine, morphine-6-glucuronide and normorphine in plasma and urine with high-performance liquid chromatography and electrochemical detection. J Chromatogr, B, Biomed Appl 1986;375:174–8.

Svensson JO, Rane A, Sawe J, Sjoqvist F. Determination of morphine, morphine-3-glucuronide and (tentatively) morphine-6-glucuronide in plasma and urine using ion-pair high-performance liquid chromatography. J Chromatogr 1982;230:427–32.

Uchihashi Y, Kuribara H, Morita T, Kitani Y, Karasawa F, Sato T. Differential effects of morphine-6-glucuronide, an active metabolite of morphine, and morphine on locomotor activity in mice: involvement of the opioid receptor. Pharmacol Toxicol 1996;78:322–6.

Wise RA, Bozarth MA. Action of drugs of abuse on brain reward systems: an update with specific attention to opiates. Pharmacol, Biochem Behav 1982;17:239–43.

Zuccaro P, Ricciarello R, Pichini S, Pacifici R, Altieri I, Pellegrini M, et al. Simultaneous determination of heroin 6-monoacetylmorphine, morphine, and its glucuronides by liquid chromatography–atmospheric pressure ionspray–mass spectrometry. J Anal Toxicol 1997;21:268–77.