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Role of Morphine Glucuronide Metabolites in Morphine Dependence in the Rat

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SALEM, A. AND W. HOPE. *Role of morphine glucuronide metabolites in morphine dependence in the rat.* PHARMACOL BIOCHEM BEHAV **57**(4) 801–807, 1997.—Concentrations of morphine and its 3- and 6-glucuronide metabolites (M3G and M6G) in plasma, brain, and urine of rats exposed to morphine for either 24 or 48 h were measured using high-performance liquid chromatography. In another group of morphine-treated rats, the intensity of naloxone-precipitated withdrawal behaviours was monitored at 24 and 48 h. The behavioural effects of M3G in opiate-naive and opiate-dependent rats were also investigated. Morphine was present in plasma, urine, and brain at 24 and 48 h, whereas M3G was detected in plasma and urine only. M6G was not present in detectable quantities in either plasma, urine, or brain. Although plasma concentrations of M3G were similar in both time groups, rats treated for 48 h had significantly larger quantities of M3G in their urine than did the other treatment groups. The incidence of withdrawal behaviour was significantly higher in animals exposed to morphine for 48 h than in those with only 24 h of exposure. M3G had no behavioural effects in the opiate-naive rats and did not precipitate an opiate-abstinence syndrome in morphine-dependent rats. From these results, it was concluded that although M3G is the major product formed by morphine breakdown in rats, it is unlikely that it is involved in the development of morphine dependence in this species. © 1997 Elsevier Science Inc.

3- and 6-Glucuronide metabolites of morphine Morphine dependence Naloxone-precipitated withdrawal

ALTHOUGH rats are most often used in laboratory studies of opiate dependence, little information is available on how this species metabolises and excretes morphine and whether or not chronically treated and dependent rats handle the drug differently from those treated acutely. This is of particular importance because morphine, which is metabolised predominantly by glucuronidation at the 3-phenolic or the 6-alcoholic hydroxyl groups, is a drug that both shows species differences in its metabolism (10,16,22,23,34) and has active metabolites (1,15,28,29).

The analgesic activity of morphine-6-glucuronide (M6G) was in fact first recognised in the early 1970s, when it was reported that the antinociceptive effect of M6G was 4–5 times that of morphine when given to mice via the subcutaneous (SC) route and 45–200 times greater when given directly into the cerebral ventricles (33). At that stage, however, M6G was considered to be present in only minute quantities following morphine treatment in humans (5), so the significance of Shimomura et al.'s (33) finding was not fully appreciated. As more sophisticated analytical techniques were developed, investigators became aware that patients given morphine for pain relief had much higher quantities of M6G present in their systems than had been previously recognised (15), thus the role of this metabolite in the pharmacological effects of morphine was reevaluated. Although there are now several lines of evidence implicating M6G in the acute effects of morphine in humans (25,26), it is not clear whether M6G is also involved in the effects of morphine in other species. In addition, whether this metabolite is involved in the development of dependence in any species has not been clarified. It is in fact possible that its role in tolerance and dependence may be significant, because not only is M6G more potent than morphine itself but it also has a longer duration of action (28).

Morphine-3-glucuronide (M3G) is reported to account for over 50% of an administered dose of morphine in humans (32), whereas in cancer patients undergoing oral morphine treatment, plasma levels of M3G are reported to be 20 times greater than morphine levels (31). Unlike M6G, M3G does not have analgesic activity in animals (28,33) nor does it have a high affinity for the μ opiate receptor (8). Smith et al. (35) suggested that rather than acting as an agonist, M3G is in fact an antagonist and that it may be involved in the development

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of tolerance to and dependence on morphine. These workers, however, based their hypothesis on the effects in rats of M3G administered exogenously and did not attempt to correlate levels of M3G following chronic morphine treatment with withdrawal behaviour. Thus, further testing is required to verify their hypothesis because, if M3G is involved in the development of tolerance and dependence, it would be expected to be found both peripherally and centrally in rats following chronic treatment with morphine. To carry out these experiments, it is necessary to determine an appropriate method for inducing opiate dependence.

Collier et al. (9) described a simple method of formulating morphine into an oil-in-water emulsion from which morphine is released slowly (slow-release emulsion; SRE). These SREs have contained morphine in the form of either its base (9) or the hydrochloride salt (40) and can be injected SC to provide a depot from which the drug is released slowly. In the present experiments, emulsions formulated with morphine hydrochloride and morphine base were compared with respect to the degree of dependence developed and the levels of morphine and its metabolites in plasma, urine, and brain. Consequently, the role of the glucuronide metabolites of morphine in the effects of this drug were also investigated.

METHODS

Animals

Experiments were carried out in female hooded Wistar rats weighing 250–300 g and housed individually in North Kent Plastics breeding cages with sawdust bedding. Standard laboratory chow and tap water were available ad lib. The room in which animals were housed was maintained at 18–20°C on a 12 L:12 D cycle.

Induction of Dependence

Morphine base (MBase) or morphine hydrochloride (MHCl) was formulated into an emulsion (saline:liquid paraffin:arlacel A at 8:6:1). The doses and treatment schedules used are shown in Table 1. The animals were divided into eight treatment groups (four groups for behavioural studies and another four groups for measurement of tissue concentrations of morphine and its metabolites) and were treated for either 24 or 48 h with an emulsion containing MBase or MHCl. When using an emulsion containing MHCl, the dosage was divided on the first day of opioid administration because pilot studies showed that a single dose of 125 mg/kg produced mortality in some animals due to marked respiratory depression. In all experiments, the total daily dose of morphine administered SC in the form of an SRE was 125 mg/kg. Each group of treated animals was compared with a control group that was administered a comparable volume of placebo or blank emulsion. All animals were both dosed and assessed for naloxone-induced withdrawal between 1100 and 1300 h to minimise potential temporal variations.

Withdrawal

Withdrawal was precipitated by administration of 3 mg/kg naloxone intraperitoneally (IP). After naloxone challenge, rats were placed into Perspex observation boxes (20 × 20 × 30 cm) for assessment of behavioural signs of withdrawal (jumps, body shakes, teeth chatter, and paw shakes) over a 20-min period. Data were expressed as the mean frequency (±SEM) of each sign per rat over the 20-min observation period. Each box was lined with preweighed paper towelling to allow collection of faecal matter during withdrawal. At the end of the observation period, the towelling was weighed and total faecal output during withdrawal was calculated.

Behavioural Effects of M3G

Animals treated with either a blank or MBase-containing emulsion were challenged with M3G. In these experiments, effects of M3G (2.5, 5, and 10 mg/kg IP) were assessed 48 h after the start of treatment with MBase-containing emulsion. Each group of opiate-dependent animals challenged with M3G was then compared with a third group of similarly treated rats challenged with saline.

Reversed-Phase High-Performance Liquid Chromatography (HPLC)

Chromatographic equipment consisted of a Rheodyne 7125 injection valve fitted with a 20-μl loop, a BAS 200A Liquid Chromatograph with a solvent delivery system, a variable wavelength UV detector, and a Phase-II ODS column (100 × 3.2 mm; BAS) with a particle size of 3 μm coupled to an RP-18 Newguard precolumn cartridge (Brownlee Labs, San Jose, CA, USA). UV absorbance of the column eluant was monitored continuously at 210 nm and recorded on a BBC SE120 dual-channel pen recorder. The mobile phase consisted of 0.02 mol/liter potassium dihydrogen orthophosphate buffer containing 19% anhydrous acetonitrile and 1-octane-sulfonic

TABLE 1

TREATMENT SCHEDULE AND TIMES OF NALOXONE WITHDRAWAL AND SAMPLE COLLECTION FOLLOWING EMULSION ADMINISTRATION

Treatment	Time of Dosing (h)	Volume of Emulsion (ml/100 g body weight)	Individual Dose (mg/kg)	Time of Naloxone Withdrawal or Sample Collection (h)
24 h MHCl	0	0.25	62.5	24
	12	0.25	62.5	
48 h MHCl	0	0.25	62.5	48
	12	0.25	62.5	
	24	0.50	125	
24 h MBase	0	0.50	125	24
48 h MBase	0	0.50	125	
	24	0.50	125	48

MHCl, an emulsion containing morphine hydrochloride; MBase, an emulsion containing morphine base.

acid (OSA) at a concentration of 10 mmol/liter as an ion-pairing agent; the pH was adjusted to 2.1 with orthophosphoric acid. The mobile phase was prepared fresh each day, filtered through 0.45-μm Millipore cellulose filters, and degassed under vacuum prior to use. The flow rate was 0.5 ml/min and temperature of the column was maintained at 28°C.

To determine linearity of the assay, standard curves (0.25–1.0 μg/ml) for morphine, its three metabolites (M3G, M6G, and normorphine), and the internal standard (IS) hydromorphone were constructed at the start of each analysis. Morphine and its three metabolites were identified on the basis of retention time and UV scanning between 190 and 360 nm.

Sample Collection

Animals were anaesthetised with halothane, and 5–7 ml of blood was collected by cardiac puncture using a 23-gauge needle and plastic Terumo syringes preloaded with 0.5 ml of 3.2% trisodium citrate. After blood samples were taken, rats were decapitated, and brains were rapidly removed and transferred to preweighed polypropylene tubes kept on ice. Brain weights were determined, 1.5 ml methanol/g tissue was added, the tissue was homogenised for 15–20 s with an Omni 1000 handheld homogeniser, and the IS was added to give a final concentration of 1 μg/ml. Samples were centrifuged for 10 min at $600 \times g$ in a Chilspin (MSE) centrifuge. The plasma and the supernatant from the brain samples were collected and stored (−4°C) until analysis.

For urine collection, animals were housed in wire cylindrical metabolism cages for a 24- or 48-h period. Food and water were available ad lib. Urine samples were transferred to preweighed polypropylene tubes, centrifuged for 10 min at $600 \times g$ in a Chilspin, and then stored (−4°C) until analysis. Sample clean-up procedures were carried out as described by Milne et al. (20). Sep-Pak $C_1$8 cartridges (Millipore-Waters) were conditioned by washing sequentially with 10 ml of methanol, 5 ml of 25% acetonitrile in 10 mM sodium dihydrogen phosphate buffer (pH 2.1), and 10 ml of HPLC-grade water at a flow rate of 5 ml/min. A 1.0-ml aliquot of either brain extract, plasma, or urine was mixed with 3.0 ml of 0.5 M carbonate buffer (pH 9.3). Immediately prior to sample extraction, urine was diluted 1:100 with HPLC-grade water. Samples were applied to the conditioned Sep-Paks at a flow rate of 2 ml/min. To remove unwanted sample components, Sep-Paks were washed sequentially at a flow rate of 2 ml/min with 20 ml of 5 mM sodium carbonate buffer (pH 9.3), 0.5 ml of HPLC-grade water, and 0.35 ml of 25% acetonitrile in 10 mM sodium dihydrogen phosphate buffer (pH 2.1). Morphine and its metabolites were eluted with 0.8 ml of 25% acetonitrile in 10 mM sodium dihydrogen phosphate buffer (pH 2.1) at a flow rate of 2 ml/min. Extracted samples were collected, and 20-μl aliquots were injected onto the HPLC column for separation and analysis on the same day. To prepare standard extracts, samples of plasma, brain, and urine obtained from placebo emulsion-treated animals were spiked with morphine and its metabolites in the range of 0.25–1.0 μg/ml and then subjected to the extraction procedure as described above.

Tissue concentrations of morphine and its metabolites were assessed at times equivalent to those when naloxone was injected to precipitate withdrawal behaviours.

Drugs and Standards

MBase was synthesised from the hydrochloride salt using the method described by Laska and Fennessy (17). MHCl was dissolved in distilled water and then sodium hydroxide (NaOH) solution (2 M) was added dropwise until a suspension of MBase began to form, as shown by the solution becoming milky, which occurred at approximately pH 6. This solution was then vacuum filtered and the precipitate was oven dried at 60°C for 4 h, weighed, and placed in a dark glass bottle for storage. Suppliers were as follows: morphine hydrochloride (Macfarlan Smith); normorphine, hydromorphone, and M3G (Alltech); potassium dihydrogen orthophosphate, sodium hydrogen carbonate, sodium hydroxide, and sodium carbonate (Ajax Chemicals, Australia); sodium dihydrogen orthophosphate and orthophosphoric acid (British Drug House); fluothane (ICI); acetonitrile and methyl alcohol chrom AR (Mallinckrodt, Australia); M6G, 1-octane-sulfonic acid, liquid paraffin, and arlacel A (Sigma, St. Louis, MO, USA). All standards were made up as a stock solution (100 μg/ml) in Millipore water and diluted to the required concentrations in the mobile phase.

Statistics

The effects of each dose of treatment drug on frequencies of behavioural signs of withdrawal were compared with controls (blank emulsion or saline challenged) using a Mann–Whitney *U*-test. Dunnetts' *t*-test was used to assess the effects of each dose of treatment drug on faecal output. The effects of duration of treatment (24 or 48 h) and morphine formulation (base or hydrochloride) on brain, plasma, and urine concentrations of morphine and its metabolites were evaluated using Students' two-tailed *t*-test. In all statistical analyses, the level of significance was taken to be 0.05.

TABLE 2

NALOXONE-PRECIPITATED WITHDRAWAL BEHAVIOUR AND FAECAL MATTER DEPOSITED BY RATS TREATED WITH MORPHINE-CONTAINING EMULSIONS

Treatment	Jumps	Body Shakes	Teeth Chatter	Paw Shakes	Faecal Matter
24 h MHCl	0	1.48 ± 0.57	1.87 ± 0.65	2.54 ± 0.95	1.60 ± 0.34
48 h MHCl	0	9.50 ± 0.91*	10.41 ± 1.14*	9.38 ± 0.92*	2.34 ± 0.25
24 h MBase	0	1.70 ± 0.4	2.50 ± 0.80	3.00 ± 0.90	0.66 ± 0.06
48 h MBase	0.45 ± 0.34	11.22 ± 1.72*	9.50 ± 0.68*	8.81 ± 1.37*	1.84 ± 0.20*

Behaviour is expressed as mean frequency ± SEM ($n = 6$), and faecal matter deposited is expressed as mean g/100 g body weight ± SEM ($n = 6$). *Significantly different ($p < 0.05$) from values observed during withdrawal following 24-h MHCl or 24-h MBase exposures.

RESULTS

Naloxone-Precipitated Withdrawal Behaviour

Within each treatment time (24 or 48 h), there were no significant differences in the frequency of any of the behavioural signs of withdrawal between rats treated with MHCl emulsion and those treated with MBase emulsion. The incidence of body shakes, teeth chatter, and paw shakes was significantly greater ($p < 0.05$, $n = 6$) in animals exposed to morphine for 48 h (base and hydrochloride) prior to naloxone challenge than in animals treated for only 24 h (Table 2). Paw shakes [3.4 ± 0.76 (mean ± SEM), $n = 6$] was the only behavioural sign observed in blank emulsion-treated rats (48 h) challenged with naloxone.

M3G-Precipitated Withdrawal Behaviour

The only two behavioural signs of opiate withdrawal seen in opiate-naive and opiate-dependent rats challenged with M3G were a low incidence of teeth chatter and paw shakes. The incidence of these two signs, however, was not significantly different from that seen in opiate-dependent animals challenged with saline (Fig. 1). A relationship between dose of M3G and severity of these two withdrawal signs was not apparent.

Amount of Faecal Matter Deposited and Changes in Body Weight Following Naloxone or M3G Challenge

With the exception of the groups of rats administered a single dose of MBase in emulsion (24 h MBase), the mean weight of faecal matter deposited following naloxone challenge was significantly greater than that recorded in the respective groups of control animals. The mean weight of faecal matter deposited following naloxone challenge was significantly greater ($p < 0.05$, $n = 6$) in animals exposed to morphine for 48 h (MBase) than in animals treated for only 24 h (Table 2). During the 20-min observation period following naloxone challenge, a significant body weight loss was recorded in morphine-treated rats.

The mean weight of faecal matter deposited following M3G challenge to morphine-treated rats was not significantly greater than in the respective group of control animals, nor was there a significant body weight loss.

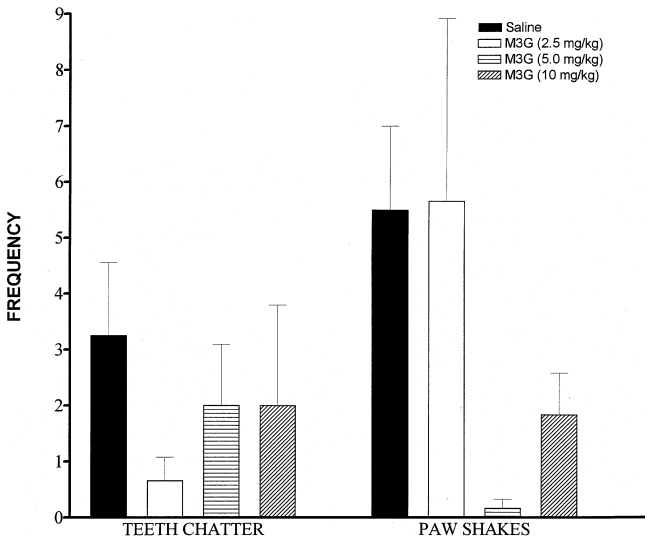

FIG. 1. Behavioural effects observed following saline or M3G challenge in rats treated with a morphine-containing emulsion (MBase, 48 h). The columns show the mean frequency (± SEM, $n = 6$) of the named behaviours observed per rat during the 20-min observation periods.

Measurement of Concentrations of Morphine and Its Metabolites

Standard curves for morphine, normorphine, M3G, M6G, and hydromorphone in the mobile phase were linear over the concentration range 0.25–1.0 μg/ml for all five compounds. Correlation coefficients between peak heights and concentrations were 0.991, 0.995, 0.994, 0.991, and 0.994, respectively. Similar results were obtained when peak areas rather than peak heights were measured. Injection of a known standard concentration indicated that the lower detection limits for morphine, M3G, M6G, and normorphine were 0.01, 0.05, 0.05, and 0.02 μg/ml, respectively.

Solid-Phase Extraction of Morphine and Its Metabolites

With the extraction procedure described in the Methods section, there was no interference from endogenous compounds present in plasma, urine, or brain samples. Extraction efficiencies [% (mean ± SEM), $n = 4$] of the sample components from rats treated with blank emulsion were as follows: M3G, 70.4 ± 3.1 (brain), 89.0 ± 3.0 (plasma), 92.1 ± 2.6 (urine); M6G, 72.4 ± 4.2 (brain), 93.2 ± 6.5 (plasma), 91.3 ± 3.7 (urine); normorphine, 79.2 ± 3.7 (brain), 84.4 ± 4.0 (plasma), 90.1 ± 6.8 (urine); morphine, 87.1 ± 4.3 (brain), 91.0 ± 5.8 (plasma), 88.0 ± 6.5 (urine), and hydromorphone 84.9 ± 7.2 (brain), 94.3 ± 3.2 (plasma), 87.3 ± 2.5 (urine).

Plasma Concentrations of Morphine and Its Metabolites

Plasma from rats treated with morphine-containing emulsions was found to contain both morphine and M3G (Table 3). Normorphine was detected at concentrations of 0.25 μg/ml in only two plasma samples. Both of these samples were obtained from rats treated for 48 h with emulsion containing MBase. No M6G was detected in plasma samples from any group of rats. Although mean plasma concentrations of M3G were consistently higher than those of morphine, there were no significant differences in plasma morphine or M3G concentrations among any of the four experimental groups of animals used.

The only statistically significant difference was between the plasma morphine concentrations in 24-h samples from rats treated with MBase-containing emulsions [0.35 ± 0.13 μg/ml (mean ± SEM), $n = 6$] vs. MHCl-containing emulsions [0.97 ± 0.05 μg/ml (mean ± SEM), $n = 6$].

Concentrations of Morphine and Its Metabolites in Brain Homogenates

Morphine was present in all brain homogenates, and there were no significant differences in the concentrations of morphine found among any of the four treatment groups (Table 3). M3G, M6G, and normorphine were not present in detectable quantities in any of the samples analysed.

Urine Concentrations of Morphine and Its Metabolites

Morphine and M3G were present in all 24- and 48-h urine samples, whereas neither normorphine nor M6G was de-

TABLE 3

PLASMA, URINE, AND BRAIN CONCENTRATIONS OF MORPHINE (MOR) AND MORPHINE-3-GLUCURONIDE (M3G) AT THE 24- AND 48-H SAMPLING TIMES

Treatment		Plasma (μg/ml)	Urine (μg/ml)	Brain (μg/g brain)
24 h MHCl	MOR	0.97 ± 0.05	326.60 ± 41.60	1.30 ± 0.20
	M3G	1.48 ± 0.29	338.33 ± 83.33	
48 h MHCl	MOR	0.92 ± 0.17	583.00 ± 83.30	1.29 ± 0.31
	M3G	1.02 ± 0.16	925.00 ± 75.00†	
24 h MBase	MOR	0.35 ± 0.13*	391.60 ± 125.00	0.80 ± 0.30
	M3G	1.29 ± 0.58	516.60 ± 50.00	
48 h MBase	MOR	0.77 ± 0.16	391.60 ± 75.00	1.01 ± 0.23
	M3G	1.33 ± 0.23	450.00 ± 58.33	

Values are mean ± SEM ($n = 6$ for plasma and brain; $n = 4$ for urine). *Significantly different ($p < 0.05$) from value in 24-h MHCl sample. †Significantly different ($p < 0.05$) from values in 24-h MHCl, 24-h MBase, and 48-h MBase samples.

tected. As in plasma samples, there were no significant differences ($p > 0.05$, $n = 4$) in urine morphine concentration in 24- or 48-h samples from rats treated with either base-containing emulsions or hydrochloride-containing emulsions (Table 3). Rats treated for 48 h with the MHCl emulsion had significantly larger ($p < 0.05$, $n = 4$) quantities of M3G in their urine than did the other treatment groups. As in the plasma samples, in all groups the mean urine concentration of M3G was greater than that of morphine. This difference, however, was significant in only the 48-h MHCl group ($p < 0.05$, $n = 4$).

DISCUSSION

Consistent with earlier reports (9,13,17,30,40), our results show that administration of morphine (base or hydrochloride) in a sustained-release emulsion produced physical dependence on the drug.

Our data also show that rats treated for either 24 or 48 h with an emulsion containing morphine (base or hydrochloride) had both morphine and M3G in plasma and urine, whereas only morphine was found in brain. With the exception of normorphine, which was present in only two plasma samples, M3G was the only metabolite detected in plasma and urine samples, thus providing support for the hypothesis that M3G is the major metabolite formed in vivo by rats (12,16,23). The concentrations of M3G in plasma were similar for the two time periods studied (24 and 48 h). In urine, the total quantity of M3G excreted over the treatment time, with the exception of rats treated for 48 h with an emulsion containing MHCl, was similar to that of morphine. In contrast, studies of the morphine metabolic pathway in humans have found that following a single intravenous dose of morphine, approximately 54–74% of urinary excretion products was M3G, whereas unchanged morphine was only 7.5–12.5% (5). It therefore appears that rats excrete considerably more unchanged morphine in their urine than do humans. This is not unexpected because, as we have mentioned before, there are quite considerable species differences in morphine metabolism. The other factor that must be considered is the difference in dosing schedules used in the two studies. Whereas our experiments involved chronic exposure to morphine, studies of morphine metabolism in humans involved administering the drug in a single dose given intravenously (5). Possibly there are differences in morphine metabolism due to prolonged exposure to the drug. In addition, it has been reported that renal clearance of M3G in rats is slower than clearance of morphine. Ekblom et al. (12) demonstrated that in rats M3G is distributed to certain tissues and is involved in an enterohepatic shunt, and other workers have shown that net tubular reabsorption occurs (39). These observations may explain the low urinary M3G:morphine ratio seen in our experiments.

In our experiments, the fact that M3G was present in the plasma in a concentration at least equal to that of morphine, and much higher than that of any other metabolite detected, suggests a possible role in the acute and chronic effects of morphine. A variety of claims have been made regarding the biological activity of M3G. This compound has been reported to be inactive as an analgesic in hot-plate and tail-flick tests (27,33), to have a low affinity for the mu-opiate receptor in receptor binding studies using rat brain homogenates (8), and to produce central nervous system excitation (hypersensitivity, tremor, wet-dog shakes, and Straub tail) in rats after intracerebroventricular or intrathecal administration (14,35). Conflicting results have been described regarding interactions between M3G and morphine. Thus, whereas Smith et al. (35) and Gong et al. (14) have reported that intracerebroventricularly administered M3G blocks the analgesic activity of morphine or M6G in the tail-flick and hot-plate tests, Suzuki et al. (36) have reported an absence of antagonistic effects of intracerebroventricularly administered M3G on morphine- or M6G-induced antinociception in the same tests.

In light of their finding that M3G blocked morphine-induced analgesia and produced some behavioural signs indicative of opiate withdrawal, it was suggested by Smith et al. (35) that M3G plays an important role in the development of tolerance and dependence to opiates. The results obtained from the present experiments, however, are not in accord with this hypothesis. If M3G were involved in the development of dependence to opiates, it is expected that it would be found both peripherally and centrally following chronic morphine treatment. Rats used in our experiments showed a high degree of dependence after 48 h of treatment with morphine and a lesser degree after 24 h, even though there was no M3G present in brain samples at either time. These data are confirmed by other workers who have also failed to detect M3G in brain following peripheral administration of morphine (11,38). Although these results suggest poor penetration of the glucuronide across the blood–brain barrier, it has been

shown that M6G and, to a lesser extent, M3G are only slightly less lipophilic than morphine (6), which enables them to penetrate into the central nervous system or cerebrospinal fluid following peripheral administration (42). Although this finding suggests that the brain concentration of M3G was too small to be determined by our assay, it is unlikely that the glucuronide metabolite was present in the central nervous system in sufficient concentrations to exert any influence on the expression of opiate withdrawal.

Furthermore, in our experiments, exogenous M3G did not produce any behavioural signs of opiate withdrawal in opiate-naive rats. Ekblom et al. (11) were also unable to demonstrate any opiate withdrawal behaviour in rats given M3G either as a bolus dose or as a constant-rate infusion. They also found that M3G caused neither antinociceptive nor hyperalgesic effects. This lack of withdrawal response in control rats given M3G is not conclusive on its own because opiate antagonists such as naloxone do not precipitate a withdrawal syndrome in opiate-naive animals (2,9,30). In opiate-dependent rats, however, administration of M3G still did not result in an opiate-abstinence syndrome. Although a low incidence of teeth chatter and paw shakes was observed in dependent rats following M3G challenge, the frequency was not significantly different from that seen in dependent animals challenged with saline. Observations of these two withdrawal behaviours in opiate-naive animals following naloxone challenge (2,9,30) further demonstrate that the behavioural signs observed in opiate-dependent rats challenged with M3G are not significant opiate withdrawal behaviour. It was of particular interest that M3G did not affect faecal output or cause weight loss during the 20-min observation period. Both of these parameters are considered to be good indicators of the severity of opiate abstinence syndrome (4,18,19,40). The increased output of faecal matter, or diarrhoea, associated with opiate abstinence syndrome is not only due to centrally mediated events (7) but also to local changes in intestinal secretions (3). Thus, it can be argued that this parameter is more susceptible to changes in plasma levels of morphine or its metabolites than are some others.

Although M6G is present in lesser quantities than M3G in humans following dosing with morphine (15,25,26,29,31,32), its marked morphinelike activity could well imply that this conjugate is of significance in terms of the biological effects produced after administration of the opioid. However, in our experiments, M6G was not detected in plasma, brain, or urine samples, suggesting that this metabolite is not formed in the rat, which is in agreement with the results of other workers, who have also failed to find any evidence for M6G production in this species (10,16,22,37). It must be remembered, however, that it is already well established that in terms of analgesia, M6G has twice the potency of morphine when administered subcutaneously and is 90–650-fold more potent than morphine when administered intracerebroventricularly or intrathecally in mice and rats (27,28,33). Given this high analgesic potency, the amount of M6G that would be required to reach the brain to produce an analgesic effect is likely to be small in comparison with morphine, possibly 90–650 times less. Because the limit of detection of our assay was 0.05 μg/ml for M6G, it is possible that enough M6G was present to induce a response, but that it was in too low an amount to be detected. There are, however, a number of arguments against this suggestion. First, because the glucuronide transfer occurs mainly in the liver, any M6G that does enter the brain can be assumed to be synthesised peripherally and to reach the central nervous system via the plasma [see review in (21)]. The analgesic potency of M6G following peripheral administration is only twice that of morphine (28), so for a response to occur, the amount of this metabolite in plasma should be high enough to account for this and should therefore be well within the detection limits of the method used. Second, the enzyme responsible for glucuronidation of morphine in the rat (UDP-glucuronide transferase) has been shown to act only at the phenolic hydroxyl moiety attached to C3 on morphine (10) and cannot transfer glucuronide to the alcoholic hydroxyl moiety on the 6-position of the morphine molecule.

The involvement of M6G in the development of dependence in any species has not been clarified. Although cross-tolerance between morphine and M6G has been demonstrated in mice made dependent on morphine (28), the only reported study of the effects of chronic treatment with M6G was of very limited scope, because only one withdrawal sign was monitored and only one dose of each compound tested was shown (22). Although these workers reported that dependence was present in mice treated with M6G, statistical analysis of these data was limited, so it is difficult to determine the significance of the results. In addition, dependence was induced by giving a total of 14 intraperitoneal injections of each drug at 8-h intervals. This procedure appears needlessly stressful and, as pointed out by Pierce and Raper (30), stress can enhance opiate withdrawal behaviour in morphine-dependent animals, which may complicate the interpretation of the results.

In summary, the data presented here show that although M3G is the major product formed by morphine breakdown in the rat, it is unlikely that it is involved in the chronic effects of morphine. There are apparent differences in the way rats and humans handle morphine, and further study is necessary to determine the relevance of results obtained from the animal model for use in studying the pharmacological effects of morphine and its metabolites in humans.

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