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Effect of Prior Morphine-3-Glucuronide Exposure on Morphine Disposition and Antinociception

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ABSTRACT. Morphine-3-glucuronide (M3G), the primary metabolite of morphine in humans and rats, has been reported to antagonize morphine-induced pharmacologic effects. The present experiment was conducted to evaluate the effect of prior systemic M3G exposure on morphine disposition and antinociceptive response in male Sprague–Dawley rats. Saline (N = 6), low dose M3G (0.15 mg/hr, N = 7), or high dose M3G (0.30 mg/hr, N = 6) was infused for 720 min prior to the administration of morphine by i.v. bolus (2 mg/kg). Tail-flick latencies in response to hot water (50°) were assessed prior to and for 180 min after the morphine test dose. M3G exposure had no significant effect on morphine pharmacokinetics, although a disproportionate increase in M3G concentrations was observed following the morphine i.v. bolus dose in rats infused with high dose M3G. Morphine-induced antinociception, expressed as the percent of maximum response (%MPR), was maximum 15 min after morphine administration and returned to baseline by 180 min. A pharmacokinetic–pharmacodynamic model was constructed to relate tail-flick latencies to morphine serum concentrations. In saline-exposed rats, the antinociceptive response to morphine was characterized by a sigmoidal E_{max} model, with an EC_{50} of 328 ng/mL, a Hill coefficient (γ) of 4.5, and a half-life for the offset of pharmacologic effect of 11 min. No statistically significant differences in the intensity or duration of morphine-induced response were detected between saline- and M3G-exposed animals. These results suggest that systemic formation of M3G is unlikely to contribute significantly to the development of tolerance to morphine antinociception. BIOCHEM PHARMACOL **53**;10: 1451–1457, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. morphine; antinociception; morphine-3-glucuronide; tolerance; pharmacodynamics; pharmacokinetics

Morphine elimination in humans is due primarily to conjugation with glucuronic acid in the liver to form M3G‡ and M6G [1, 2]. During chronic administration of oral morphine, both glucuronide metabolites accumulate to a greater extent than morphine, with a metabolite-to-parent AUC ratio of 34.0 and 3.9 for the 3- and 6-glucuronides, respectively [3]. Due to their unusual lipophilicity [4], morphine glucuronides may be able to cross the blood–brain barrier [5, 6] and produce pharmacologic effects in the central nervous system. While M6G possesses potent analgesic properties and may contribute to morphine analgesia [5, 7, 8], M3G has been reported to antagonize the effects of morphine and M6G and, potentially, to play a role in the development of tolerance (i.e. loss of antinociceptive response at a fixed systemic morphine concentration during continuous morphine administration) [9–11]. However, existing studies are limited by the fact that high systemic concentrations of M3G were utilized, or the metabolite was administered directly into the central nervous system. Neither situation mimics the delivery of M3G to the brain after systemic formation from morphine.

The objective of the present experiment was to evaluate the effect of prior M3G exposure on morphine disposition and antinociceptive response in rats. Rats were exposed to M3G for a period of 12 hr prior to the morphine test dose, since tolerance has been shown to develop within that time period during constant morphine infusion [12, 13]. The rat was selected as an animal model due to its similarity to humans in terms of morphine metabolism, with the major elimination route being the formation of M3G. An additional advantage of this animal model is the absence of M6G formation [14, 15], which would complicate interpretation of the dynamic response.

MATERIALS AND METHODS

Animals

Experiments were performed with male Sprague–Dawley rats (Hilltop Laboratory Animals Inc., Scottdale, PA)

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‡ *Abbreviations:* M3G, morphine-3-glucuronide; M6G, morphine-6-glucuronide; %MPR, percent of maximum possible response; AUE, area under the effect–time curve; AUC, area under the concentration–time curve; $Peak_{obs}$, observed peak effect; T_{peak}, time to observed peak effect; and PK–PD, pharmacokinetic–pharmacodynamic.

weighing between 290 and 365 g. All rats were housed in temperature-controlled rooms with a 12-hr light/day cycle, and were acclimated for a minimum of 5 days prior to experimentation. Implantation of silicone rubber cannulae in the right jugular and right femoral veins was performed under light ether anesthesia the day prior to the experiment. Rats were allowed free access to food and water throughout the experimental period. All procedures involved in the animal experimentation were approved by the Institutional Animal Care and Use Committee of The University of North Carolina at Chapel Hill.

Materials

Morphine (as the sulfate salt; Research Biochemicals Inc., Natick, MA) and M3G (Sigma Chemical Co., St. Louis, MO) were dissolved in normal saline for administration. Nalorphine hydrochloride was purchased from the Sigma Chemical Co. as an analytical standard. All reagents and solvents were obtained from commercial sources.

Experimental Design

On the day of the study, low dose M3G (0.15 mg/hr; N = 7), high dose M3G (0.30 mg/hr; N = 6), or normal saline (N = 6) was infused through the femoral vein cannula (1 mL/hr) for the duration of the experiment. After 720 min of exposure, morphine was administered as a 2 mg/kg i.v. bolus. Antinociceptive effect was evaluated prior to the M3G infusion (−720 min) and immediately prior to (0 min) and at timed intervals after (7.5, 15, 30, 45, 60, 120, and 180 min) the morphine bolus dose. Rats exposed to normal saline (N = 2) or M3G (N = 3) but not receiving morphine were included as additional controls to evaluate baseline antinociception throughout the experiment. Antinociception was measured with the standard hot water-induced tail-flick test. Rats were placed in Plexiglas restraining cages 30 min prior to tail-flick testing. The distal 5 cm of the tail was immersed in water (50°), and the time to withdrawal of the tail was measured in duplicate. A cutoff time of 15 sec was used to minimize tissue damage. Antinociception was expressed as percent of maximum possible response (%MPR):

$$\%\text{MPR} = \frac{\text{Test latency} - \text{Baseline latency}}{\text{Cutoff time} - \text{Baseline latency}} \cdot 100 \quad (1)$$

Blood (0.15 to 0.30 mL) was withdrawn from the jugular vein immediately after pharmacologic assessment, and two additional blood samples were taken at 3 and 90 min post-dose. Samples were centrifuged for 10 min, and the serum was harvested and stored at −20° pending analysis.

Morphine and M3G Analysis

Serum morphine and M3G concentrations were quantitated with a sensitive and specific HPLC method with fluorescence detection described previously [16]. Briefly, serum samples (50–200 μL) in which nalorphine hydrochloride was added as an internal standard were alkalinized with ammonium sulfate buffer (pH 9.3) prior to solid-phase extraction. Following recovery of the analyte molecules by elution with methanol, the eluent was evaporated to dryness, and the residue was reconstituted in the mobile phase (10% acetonitrile in trifluoroacetic acid, 0.1% aqueous solution) prior to injection onto the HPLC system. Chromatographic separation was achieved on a reverse-phase column with constant-flow (1 mL/min) gradient elution (increasing acetonitrile content from 7 to 14% in 0.1% trifluoroacetic acid). Fluorescence of the column eluent was monitored continuously at an excitation wavelength of 214 nm and an emission cutoff of 370 nm. Under these conditions, the retention times for M3G, morphine, and the internal standard were 5, 8, and 15 min, respectively. Standard curves constructed in blank rat serum were linear ($r^2 > 0.999$) up to 3000 ng/mL, with a detection limit of 37.5 ng/mL. The assay was reproducible, with intra-day coefficients of variation for morphine and M3G of 4.9 and 7.2%, respectively.

PK–PD Analysis

Morphine concentrations in a central compartment (volume = V_c) were described by either 1 or 2 pharmacokinetic compartments with first-order elimination from the central compartments (k_{10}) and intercompartmental flux according to the usual distribution rate constants (k_{12}, k_{21}). A separate effect compartment was necessary to account for the delay observed in onset of antinociception relative to the pharmacokinetic profile. The effect compartment was linked to the central compartment by a first-order rate constant (k_{1e}); disappearance from the effect compartment was characterized by the first-order elimination rate constant k_{e0}. The effect compartment model, originally developed by Sheiner *et al.* [17], requires the following assumptions: (1) first-order processes govern the onset and offset of pharmacologic effect; (2) the amount of drug distributing to the effect compartment is negligible relative to the total body load of the drug; and (3) clearances into and out of the effect compartment are equal so that $(V_c \cdot k_{1e}) = (V_e \cdot k_{e0})$. Antinociceptive effect was related to morphine concentrations in the effect compartment (C_e) with the following sigmoidal E_{max} equation:

$$\%\text{MPR} = \frac{E_{max} \cdot C_e^{\gamma}}{EC_{50}^{\gamma} + C_e^{\gamma}} \quad (2)$$

where E_{max} is defined as the maximum response, EC_{50} is the concentration in the effect compartment producing 50% of E_{max}, and γ is the Hill coefficient describing the shape of the concentration–response curve.

The relevant pharmacokinetic and pharmacodynamic parameters were generated for each rat by nonlinear least-squares regression analysis (PCNONLIN version 3.0; Sta-

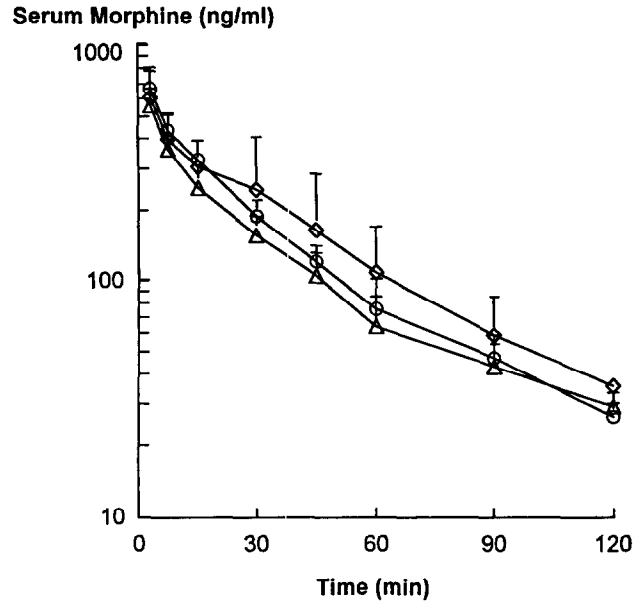

FIG. 1. Morphine serum concentration versus time profiles after a 12-hr infusion of saline (○; N = 6), low-dose M3G (△; N = 7), or high-dose M3G (◇; N = 6). Data are presented as means ± SD. Estimates of pharmacokinetic parameters are compiled in Table 1.

tistical Consultants Inc., Apex, NC) and by standard noncompartmental methods. The AUC and the AUE were calculated for each rat with the linear trapezoidal method. Parameter estimates were obtained by simultaneous fitting of the effect versus time and morphine concentration versus time data. Assessment of the goodness of fit of the model to the observed data was based on Akaike's Information Criterion (AIC), residual plots, coefficients of determination, and standard error of the estimates.

Differences in parameter estimates among the three experimental groups were tested for statistical significance with ANOVA. Statistical significance was defined as a P value < 0.05. All parameter estimates are reported as means ± SD.

RESULTS

Mean serum morphine and M3G concentration versus time profiles are presented in Figs. 1 and 2, respectively. Morphine concentration–time profiles were almost superimposable between the saline-, low dose M3G-, and high dose M3G-exposed rats, with an elimination half-life of approximately 13 min. Although mean morphine concentrations appeared slightly greater starting 30 min post-dose in the high dose M3G-exposed rats, no statistically significant differences were observed among the groups for any of the morphine pharmacokinetic parameters. The pharmacokinetic parameter estimates are summarized in Table 1. A two-compartment pharmacokinetic model was necessary to describe the serum morphine concentration–time profile in two of the saline-, four of the low dose M3G-, and two of the high dose M3G-exposed rats; a one-compartment system was sufficient for the remaining animals in the experiment. Based on the steady-state M3G concentrations observed after 720 min, mean ± SD systemic clearances of the preformed metabolite were 13.1 ± 2.8 and 14.8 ± 1.6 mL/min/kg in the low and high M3G dose groups, respectively. No morphine was detected in serum during M3G infusion.

Following administration of morphine in saline-treated rats, conversion to the metabolite resulted in peak M3G concentrations of 530 ng/mL, with a mean ± SD AUC ratio of parent to metabolite (corrected for differences in molecular weight using a 1.6174 ratio) of 1.3 ± 0.6. An estimate of the fraction of morphine metabolized to M3G was obtained by calculating the metabolite AUC obtained after parent administration in the saline-exposed rats and using the systemic clearance of M3G determined from steady-state M3G concentrations. Based on these results, about 22% of morphine was converted to M3G over the 180-min experiment. Disproportionate increases in M3G maximum concentration (C_{max}) and AUC were observed following the morphine i.v. bolus dose in the high dose M3G-exposed group, even when steady-state M3G concentrations were taken into consideration.

Tail-flick latencies remained constant throughout the experiment in control rats not receiving morphine. The mean ± SD AUE for control animals (N = 3) was −714 ± 1308 %MPR · min, which was not statistically different from 0. In treated rats, the infusion of M3G had no effect on the antinociceptive response: the difference in effect between the beginning and the end of the M3G infusion was −1.83 ± 6.48 %MPR and −1.35 ± 6.92 %MPR in the low and high M3G dose groups, respectively. The difference between the start and end of the saline infusion was +1.62 ± 5.83 %MPR.

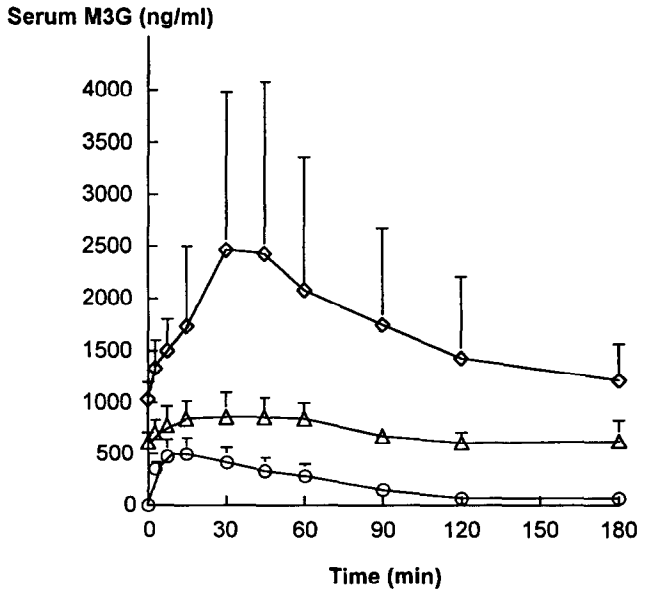

FIG. 2. M3G serum concentration versus time profiles after a 12-hr infusion of saline (○; N = 6), low-dose M3G (△; N = 7), and high-dose M3G (◇; N = 6). Data are presented as means ± SD.

TABLE 1. Noncompartmental and model-dependent pharmacokinetic parameter estimates (mean ± SD) for morphine and M3G

Parameter	Saline (N = 6)	Low dose M3G (N = 7)	High dose M3G (N = 6)
Morphine			
V_c (L/kg)	2.69 ± 1.07	2.60 ± 0.98	3.09 ± 1.33
k_{10} (min^{-1})	0.0511 ± 0.0223	0.0584 ± 0.0278	0.0513 ± 0.0378
k_{12}* (min^{-1})	0.179 ± 0.169	0.136 ± 0.090	0.0871 ± 0.0193
k_{21}* (min^{-1})	0.112 ± 0.081	0.0932 ± 0.0250	0.0831 ± 0.0479
CL† (mL/min/kg)	108 ± 20	121 ± 21	103 ± 35
V_{ss}† (L/kg)	3.72 ± 0.52	4.91 ± 1.47	4.34 ± 1.86
MRT† (min)	35.3 ± 7.9	42.8 ± 7.4	41.9 ± 6.8
M3G			
Total C_{ss} (μg/mL)	NA‡	0.611 ± 0.111	1.04 ± 0.16
Total C_{max} (μg/mL)	0.531 ± 0.161	0.980 ± 0.221	2.63 ± 1.59
Total AUC_{m3g} (μg · min/mL)	36.8 ± 11.7	123 ± 22	306 ± 155
Derived C_{max}§‖ (μg/mL)	0.531 ± 0.161	0.369 ± 0.124	1.59 ± 0.80
Derived AUC_{m3g}§‖ (μg · min/mL)	36.8 ± 11.7	13.0 ± 14.9	120 ± 79

* Intercompartmental rate constants were determined in two saline-, four low dose M3G-, and two high dose M3G-exposed rats.
† CL, V_{SS}, and MRT were determined using noncompartmental analysis.
‡ NA: not applicable.
§ Calculated as the difference between total C_{max} (or total AUC_{m3g}) and C_{ss} (or AUC_{m3g} · 180 min).
‖ $P < 0.005$ (ANOVA).

Mean antinociceptive effect versus time profiles for saline- and M3G-exposed groups are presented in Fig. 3. The pharmacodynamic parameter estimates are summarized in Table 2. Following morphine i.v. bolus in saline-exposed rats, the antinociceptive response was maximal 15 min post-dose, and returned to baseline by 120–180 min post-dose. The $Peak_{obs}$ was not affected by M3G; mean peak tail flick latencies ranged from 51.4 to 64.8 %MPR among the three experimental groups. Peak effects were observed between 7.5 and 45 min post-dose (T_{peak}). The effect

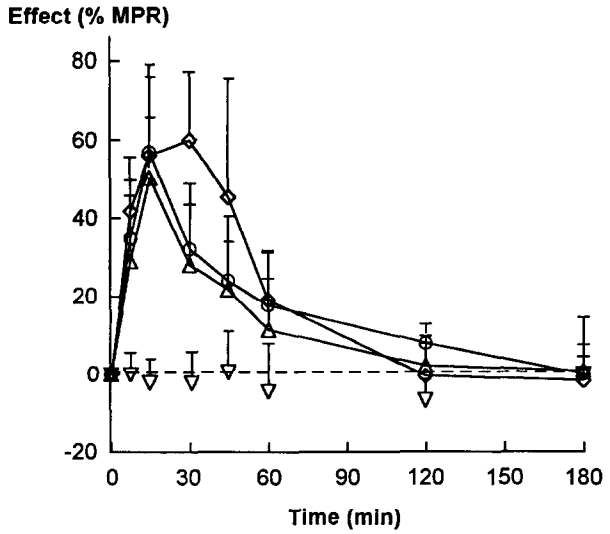

FIG. 3. Antinociceptive effect versus time profiles in saline (○; N = 6), low-dose M3G (△; N = 7), and high-dose M3G (◇; N = 6) exposed rats. Also shown are data from control animals not receiving the morphine bolus (▽; N = 5). Data are presented as means ± SD. Estimates of pharmacodynamic parameters are compiled in Table 2.

appeared to be more sustained in the high dose M3G-treated rats compared with the two other groups. This difference may be explained by the larger morphine concentrations observed in that group starting at 30 min post-dose. Indeed, when normalized for differences in morphine concentration, the ratios of AUE over morphine AUC were similar among the groups (Table 2). The sigmoid E_{max} model with effect compartment described well the data in the three groups, with mean EC_{50} values ranging from 252 to 369 ng/mL, mean γ ranging from 3.0 to 4.5, and half-life for the effect compartment of 9–12 min. No statistically significant differences were observed between groups in any of the pharmacodynamic parameters estimated from the PK–PD model. Individual tail flick data are plotted against predicted morphine concentrations in the effect compartment in Fig. 4. The effect versus concentration relationship did not differ between treatment groups, further indicating a lack of effect of M3G on morphine dynamics.

DISCUSSION

Previous experiments in this laboratory quantitated morphine-induced antinociception with hot-water induced tail-flick during a 12-hr constant-rate i.v. infusion [12]. During morphine infusion, maximal effect was achieved after 180 min and decreased thereafter despite sustained morphine concentrations. This observation suggests the development of significant tolerance, which was nearly complete at the end of the 12-hr infusion. A PK–PD model was constructed to describe the dynamic response relative to morphine concentrations. Antinociceptive response to morphine was characterized by an EC_{50} of 389 ng/mL, a Hill

TABLE 2. Pharmacodynamic parameter estimates (mean ± SD) based upon PK–PD modeling and non-compartmental analysis of the tail-flick and concentration–time data

Parameter	Saline (N = 6)	Low dose M3G (N = 7)	High dose M3G (N = 6)
E_{max}*† (%MPR)	100	100	100
EC_{50}† (ng/mL)	328 ± 161	369 ± 231	252 ± 95
γ†	4.53 ± 3.92	4.48 ± 4.91	3.01 ± 2.79
k_{e0}† (min^{-1})	0.0657 ± 0.0269	0.0566 ± 0.0286	0.0765 ± 0.0367
$Peak_{obs}$ (%MPR)	56.8 ± 19.2	51.4 ± 27.2	64.8 ± 15.9
T_{peak} (min)	15.0 ± 0.0	21.4 ± 11.8	26.3 ± 16.3
AUE_{180} (%MPR · min)	2638 ± 1007	2116 ± 1367	3159 ± 762
AUE_{180}/AUC_{180} (%MPR/ng/mL)	0.180 ± 0.070	0.157 ± 0.076	0.188 ± 0.083

* E_{max} was fixed as the theoretical maximum, and was not determined as a parameter in analyzing the tail-flick data.
† Parameter was obtained from the fit of the PK–PD model to tail-flick and serum morphine concentration data.

coefficient (γ) of 2.64, and a half-life for response onset of 9 min. These results are in reasonable agreement with those obtained in the present experiment in which morphine was administered as an i.v. bolus. The PK–PD model for morphine tolerance incorporated a hypothetical partial agonist with a high affinity and low intrinsic activity, the accumulation of which was responsible for tolerance development. Concentrations of the partial agonist were linked to systemic morphine concentrations by a first-order rate constant, consistent with a biotransformation product formed under linear pharmacokinetic conditions.

Since M3G has been implicated as a morphine antagonist, the present experiment was conducted to evaluate the potential contribution of M3G to the development of tolerance by assessing whether prior M3G exposure reduced the intensity and/or duration of pharmacologic response to acute administration of morphine. To ensure optimal development of potential tolerance, M3G was administered as a continuous i.v. infusion for 12 hr prior to administration

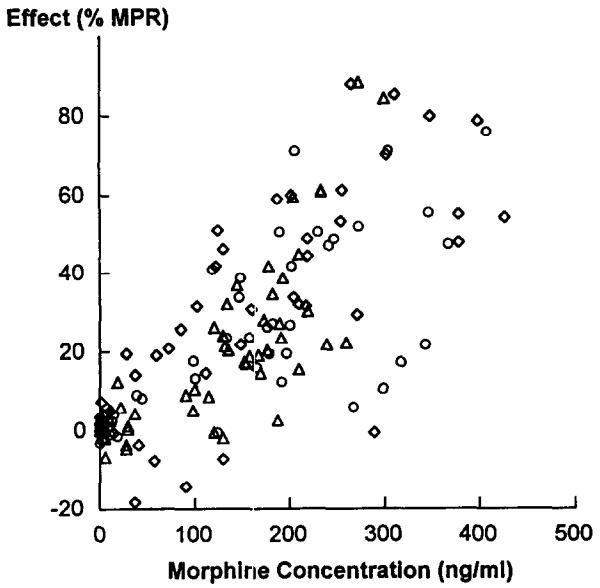

FIG. 4. Antinociceptive effect versus calculated morphine concentrations in the effect compartment in saline (○)-, low-dose M3G (△)-, and high-dose M3G (◇) exposed rats.

of the morphine test dose. As discussed above, a similar morphine infusion regimen has been shown to produce significant tolerance; the tail flick response 15 min after administration of a 2 mg/kg test dose of morphine in tolerant rats (12 hr after cessation of a 12-hr 2 mg/kg/hr morphine infusion) was only 25.7 ± 9.0% (N = 3) of the response measured in naive rats after a 2 mg/kg morphine bolus. Therefore, if M3G formation from morphine contributed significantly to the development of tolerance, a similar decrease in peak effect would have been expected in the present experiment.

M3G concentrations observed after 12 hr of infusion were within the range of M3G concentrations measured during administration of morphine at a rate of 2 mg/kg/hr [12]. The results reported herein demonstrated that systemic administration of M3G for 720 min had no effect on either baseline tail-flick latency or tail-flick response to morphine. These results are consistent with those of Hewett *et al.* [18] who showed that intrathecal M3G did not possess antinociceptive activity and did not decrease the antinociceptive response to morphine. Other investigators have reported that administration of M3G directly to the central nervous system results in a significant reduction in pharmacologic response to morphine or M6G [10, 11]. The mechanism of the putative interaction between M3G and morphine is unknown, since M3G binds poorly to opioid receptors [8].

Even though some data regarding the effect of centrally administered M3G on morphine antinociception supports the hypothesis that M3G is a morphine antagonist, the influence of systemic M3G (i.e. relevant to the situation in which M3G is formed from morphine) on analgesic response remains unclear. Using a cross-over design, Smith *et al.* [10] administered intraperitoneal morphine either alone or 30–40 min after M3G in rats, and reported a significant reduction in morphine-induced antinociception. However, only a 5- to 7-day washout period between treatments was used, and the possibility of a carry-over effect was not tested. Ekblom *et al.* [9] reported lower tail-flick latencies to i.v. bolus morphine (10 mg/kg) in rats exposed to a 4-day i.v. infusion of M3G (1.4 mg/kg/hr) than in control

animals. Morphine could be detected in serum during the M3G infusion; the presence of morphine may have caused the development of tolerance, thereby contributing to the decrease in dynamic response observed after the test dose on day 4. No attempt was made to relate the antinociceptive response to systemic morphine concentrations.

High doses of M3G administered directly to the spinal cord produced a hyperalgesia that apparently was not mediated by opiate receptors [19]. Thus, apparent antagonism of morphine by high-dose M3G may represent central hyperexcitability that would not occur with M3G derived from typical doses of morphine. In contrast to these studies, Lipkowski *et al.* [20] reported that acute administration of M3G actually potentiated the effects of morphine. These authors administered a large (>9 mg/kg) bolus dose of M3G in conjunction with morphine, and reported a statistically significant elevation of antinociception as compared with rats receiving saline plus morphine. However, morphine concentrations were not determined, and the possibility that M3G altered morphine concentrations at the site of action, either through systemic or local inhibition of morphine clearance or distribution, cannot be discounted. In the present experiment, morphine-induced antinociception was characterized with a PK–PD modeling approach, and no changes in dynamics could be detected in M3G-exposed rats with pharmacologically relevant systemic concentrations of M3G.

In an interesting and relevant study, Smith and Smith [21] examined the degree of antinociceptive tolerance to morphine in rats relative to circulating concentrations of morphine and derived M3G. Rats received differing morphine infusion regimens to produce different morphine blood concentration versus time profiles over 96–192 hr. A statistically significant inverse relationship was observed between antinociceptive effect and the M3G:morphine plasma concentration ratio. These data suggest that the pharmacologic activity of morphine decreases as circulating concentrations of M3G (relative to morphine) increase. However, due to the slow accumulation of M3G during morphine infusion, as well as the manner in which morphine was administered, the M3G:morphine concentration ratio increased with time in all treatment groups. Similarly, the antinociceptive action of morphine decreased with time in all groups due to the onset and progression of tolerance. Thus, the apparent inverse relationship between antinociceptive activity and systemic M3G concentrations may have been an artifact of the pharmacodynamics (time-dependent decrease in activity, irrespective of M3G concentration) and the pharmacokinetics (time-dependent increase in the M3G:morphine ratio due to slow M3G accumulation) of this system, rather than a cause–effect relationship between systemic M3G and loss of pharmacologic activity.

A secondary objective of the present experiment was to assess the effect of prior M3G exposure on morphine and M3G disposition. The M3G clearance reported herein was similar to previously reported results after administration of M3G as an i.v. bolus [16]. In general, pretreatment with M3G did not affect morphine disposition. Concentrations of derived M3G were higher in the high dose M3G-exposed group compared with the concentrations of the derived metabolite observed in saline- and low dose M3G-exposed rats (Table 1). It has been reported that M3G is eliminated via urinary excretion (56% of dose administered) and, like morphine, undergoes enterohepatic recirculation [16, 22, 23]. During exposure to high dose M3G, competition for biliary transport with parent drug may occur, resulting in a larger fraction of the morphine dose being converted to M3G. Alternatively, the biliary and/or renal excretion of M3G may be capacity-limited, resulting in higher concentrations of derived M3G in rats exposed to high concentrations of preformed metabolite.

In summary, no significant pharmacokinetic or pharmacodynamic interactions between M3G and morphine could be documented in a rat tail-flick model of antinociception. These data suggest that a significant contribution of systemically formed M3G to morphine pharmacodynamics, including tolerance development, is unlikely.

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