Blood-Brain Barrier Permeability to Morphine-6-Glucuronide is Markedly Reduced Compared with Morphine

ABSTRACT:

The blood-brain barrier (BBB) permeability to morphine and morphine-6-glucuronide (M6G) is measured under identical conditions using an intravenous injection method in the rat and HPLC separation of morphine from its metabolites. The brain uptake of M6G expressed as %ID/g was 32-fold lower than that of morphine, and the BBB permeability surface area product (PS) of M6G was 57-fold lower as compared with that of morphine. Consistent with these in vivo data, the 1-octanol/buffer partition study showed the liposolubility of M6G was 187-fold lower than that of morphine. The CNS origin of M6G analgesia after peripheral administration was confirmed because the analgesia was completely blocked by naloxone, which crosses BBB, but not by naloxone methiodide, which does not enter brain from blood. In conclusion, the BBB permeability to M6G is markedly reduced as compared with morphine, consistent with the much lower lipid solubility of M6G relative to morphine.

M6G\(^1\) is an active metabolite of morphine (1). Analgesia studies in experimental animals (2, 3) demonstrated that, although M6G and morphine were almost equally potent after peripheral administration, the analgesic potency of M6G was >100-fold higher than morphine after intracerebroventricular injection, a route of administration that bypasses the BBB in vivo. These pharmacological data suggest that the brain penetration of M6G is significantly attenuated relative to that of morphine, probably due to the attachment of a glucuronide moiety to M6G and its high hydrophilic property. However, several studies using microdialysis fibers (4–6) showed that the brain interstitial fluid AUC for both morphine and M6G was comparable after peripheral administration. In another study that used arterial-venous sampling in pigs, the net cerebral extraction of morphine and M6G was reported to be 50–58% for either compound (7). By contrast, Bickel et al. (8) have recently shown that the BBB permeability to morphine is ~30-fold greater than M6G after internal carotid artery perfusion.

In view of the conflicting data in the literature regarding the brain penetration of M6G, the present studies were designed to measure BBB permeability to morphine and M6G under identical conditions using a conventional intravenous injection/pharmacokinetic method in the rat. Because morphine is extensively converted to M3G in the rat (9), the present studies used HPLC separation of plasma radioactivity so that accurate measurements of the morphine AUC could be determined. In addition to the pharmacokinetic analysis and measurement of BBB transport for both morphine and M6G, the lipid solubility of these two compounds was also measured in parallel using the 1-octanol/aqueous partition method (10). Finally, the CNS origin of M6G analgesia was demonstrated after peripheral administration of the compound by comparing the antagonistic effects of naloxone, which crosses the BBB, and naloxone methiodide, which does not enter brain from blood (11).

Materials and Methods. Male Sprague-Dawley rats (250–350 g) were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). \(^{[3}\text{H}]\text{Morphine} (86.5 \text{ Ci/mmol})\) was purchased from DuPont NEN Products, Inc. (Boston, MA). \(^{[3}\text{H}]\text{M6G} (21.4 \text{ Ci/mmol})\) was synthesized by the Triangle Research Institute (Research Triangle Park, NC). The radioactive purity of \(^{[3}\text{H}]\text{M6G}\) was reported to be 98% by an HPLC analysis, and this was confirmed in our TLC radioscanning assay. Morphine sulphate, M3G, M6G, and naloxone HCl were supplied by Sigma Chemical Co. (St. Louis, MO). Naloxone methiodide was purchased from Research Biochemical International (Natick, MA). All other chemicals were of analytical grade and were obtained from Fisher Scientific (Tustin, CA).

Intravenous Administration. Rats were anesthetized with 100 mg/kg ketamine and 2 mg/kg xylazine intraperitoneally. The left femoral vein was cannulated with a PE-50 cannula and injected with 0.2 ml Ringer-HEPES solution (pH 7.4) containing either 5 \(\mu\text{Ci}\) (0.058 nmol) of \(^{[3}\text{H}]\text{morphine}\) or 5 \(\mu\text{Ci}\) (0.23 nmol) of \(^{[3}\text{H}]\text{M6G}\). Blood samples (0.25 ml) were collected via heparinized PE-50 cannula implanted in the left femoral artery at 0.25, 0.5, 1, 2, 5, 10, 30, or 60 min after the injection. Blood volume was replaced with an equal volume of saline. Animals were killed at 60 min after the intravenous injection for removal of the brain. Plasma and organ samples were solubilized with Soluene-350 (Packard Instrument Company, Downer’s Grove, IL) and neutralized with glacial acetic acid before liquid scintillation counting.

HPLC Analysis. Extraction of \(^{[3}\text{H}]\text{morphine}\) and \(^{[3}\text{H}]\text{M6G}\) from the plasma was conducted by the method reported by Milne et al. (12). Briefly, plasma (0.1 ml) was made alkaline by the addition of 3 ml of 500 mM NaHCO\(_3/\)Na\(_2\)CO\(_3\) buffer (pH 9.3), and applied to Sep-Pak C\(_{18}\) cartridge (Waters Co. Milford, MA), which was activated by rinsing successively with 10 ml of methanol, 5 ml of 25% acetonitrile/10 mM NaH\(_2\)PO\(_4\) buffer (pH 2.1), and 5 ml of H\(_2\)O. After being
washed with 20 ml of 5 mM NaHCO<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub> buffer (pH 9.3), 0.5 ml of water, and 0.35 ml of 25% acetonitrile in 10 mM NaH<sub>2</sub>PO<sub>4</sub> buffer (pH 2.1), the sample was eluted with an additional 0.8 ml of the acetonitrile/NaH<sub>2</sub>PO<sub>4</sub> buffer, and 0.25 ml of the collected sample was enriched with 1 μg of each morphine, M3G and M6G, for HPLC analysis.

HPLC assay of morphine and its metabolites was performed using a Perkin-Elmer system consisting of two pumps (model 410 BIO), an analytic C<sub>18</sub> column (4.6 × 150 mm), a C<sub>18</sub> precolumn (3.2 × 15 mm), an ultraviolet detector (model LC-90 BIO) set at 210 nm, and an integrator (model LCL-100). Samples were eluted isocratically with a mobile phase consisting of 26.5% acetonitrile and 73.5% 0.8 mM of 1-dodecysulfate sodium in 10 mM of NaH<sub>2</sub>PO<sub>4</sub> buffer (pH 2.1) at a flow rate of 0.8 ml/min. Fractions (0.8 ml) were collected using a Pharmacia fraction collector (model FRAC-100), and the radioactivity of each fraction was counted. M3G, M6G, and morphine were well separated on the column. Their retention times were 5.07, 6.48, and 9.03 min, respectively. The recoveries of [3 H]morphine (1 μCi) and [3 H]M6G (1 μCi) from control rat plasma were 76.9 ± 2.6% (N = 6) and 69.4 ± 3.2% (N = 6), respectively.

**Pharmacokinetic Analysis.** Pharmacokinetic parameters were calculated by fitting the plasma radioactivity data to a biexponential equation:

\[ A(t) = A_1 e^{-K_{12}t} + A_2 e^{-K_{21}t}, \]

where \( A(t) = \%ID/ml \) plasma. For rats injected with [3 H]morphine, the total plasma radioactivity was multiplied by the [morphine/(morphine + M3G)] ratio, where the morphine and M3G fractions were determined by HPLC. The ratio was 0.98, 0.94, 0.86, 0.74, 0.60, 0.55 and 0.55 at 0.25, 1, 2, 5, 15, 30, and 60 min after injection, respectively. No significant [3 H]M6G peak was observed in plasma obtained from rats treated with [3 H]morphine, which confirms previous studies that morphine is quantitatively converted to M3G in the rat (9). The biexponential equation was fit to plasma data using a derivative-free nonlinear regression analysis (PARBMDP, Biomedical Computer P-Series, developed at UCLA Health Sciences Computing Facilities). Data were weighted using weight = 1/(concentration)<sup>2</sup>, where concentration = %ID/ml plasma. The organ volume of distribution, BBB PS product, and the brain uptake (%ID/g) of [3 H]morphine and [3 H]M6G at 60 min after intravenous injection, and the pharmacokinetic parameters such as plasma clearance, the initial plasma volume, systemic volume of distribution, steady-state AUC (AUC<sub>ss</sub>), and mean residence time were determined from the A<sub>1</sub>, A<sub>2</sub>, K<sub>12</sub>, and K<sub>21</sub>, as described previously (13, 14).

**P and Protein Binding Studies.** The lipid solubility of [3 H]morphine and [3 H]M6G was determined with the 1-octanol/buffer partition method as described previously (10). Plasma protein binding of [3 H]M6G (10 nM or 0.33 μCi/ml) in 4% HSA (w/v), 4% RSA (w/v), or 100% rat serum was assessed by an equilibrium dialysis as described previously (10).

**Analgesia Testing.** The analgesic effects of M6G were measured in rats according to the method described by Sami et al. (15). Briefly, rats were allowed 30 min adaptation in the testing room before drug treatments. After three baseline tail-flick measurements, all of the three groups of rats (3 rats per group) received 5 mg/kg of M6G (10 μmol/kg sc). A tail-flick test was conducted up to 4.5 hr postdose. At 30 min, group 1 received a second injection of corresponding volume of saline as control, whereas groups 2 and 3 were given 3.7 mg/kg of naloxone HCl (10 μmol/kg sc), and 4.7 mg/kg of naloxone methiodide (10 μmol/kg sc), respectively. This dose of methyl naloxone antagonizes peripheral, but not central, pharmacological effects of morphine in rats (11).

**Results and Discussion.** Pharmacokinetics. As shown in fig. 1, the [3 H]morphine radioactivity in the rat plasma expressed as %ID/ml after intravenous injection decreased in a biexponential manner. HPLC analysis revealed a time course of the formation of M3G, and no M6G was detected. The plasma profile of [3 H]M6G was compared with that of [3 H]morphine after extraction and HPLC analysis, and no morphine peak was detected after administration of M6G. The distribution phase of [3 H]morphine was faster than that of [3 H]M6G, whereas the terminal curves of the two compounds were parallel. The pharmacokinetic parameters for [3 H]morphine and [3 H]M6G estimated from the plasma profile data (fig. 1) are listed in table 1. The AUC value for M6G at 60 min after administration was 1.8-fold higher than the AUC for morphine (18.2 ± 0.9 vs. 10.1 ± 0.4 %ID min/ml). The volume of distribution and clearance of morphine was nearly 2.5-fold greater than those of M6G. Finally, the terminal half-lives of morphine and M6G were in the same range (37 ± 3 vs. 36 ± 13 min) during a 60-min period of observation. These results are consistent with the clinical pharmacokinetic studies of morphine and M6G by Hanna et al. (16), who reported that M6G is more metabolically stable than morphine, with a greater AUC value after an intravenous injection, but the elimination half-lives of the two compounds are similar in human subjects.

**BBB Permeability and Brain Uptake.** Despite the greater AUC value for M6G as compared with morphine, the brain uptake of M6G expressed as %ID/g was 32-fold lower than that of morphine (table 1). This is because the brain volume of distribution of M6G was 22-fold lower than that of morphine. Consistently, the PS product of M6G was 57-fold lower than that of morphine (0.142 ± 0.026 vs. 8.03 ± 0.28 μl/min/g), an observation that parallels those reported by Bickel et al. (8) in the internal carotid artery perfusion study.

**P and Protein Binding.** The P of morphine has been determined by many groups [see a recent review by Milne et al. (17)]. With respect to the P of M6G, Carrupt et al. (18) reported that the log K<sub>b</sub> for morphine is greater than the log K<sub>b</sub> for M6G, as determined by HPLC. Measurements of the lipid solubility of these two compounds by the 1-octanol/Ringer-HEPES buffer (pH 7.4) partition method reported herein show a 187-fold difference between the P of morphine and M6G (table 1), which yields log P values of −0.69 and −2.95 for morphine and M6G, respectively. The P of morphine derived from
this study conducted at room temperature is close to the value reported by Poyhia and Seppala (19), who used an octanol/Tris-HCl method at 37°C.

The equilibrium dialysis study revealed that the percentage of free \(^{[3}H\)M6G in 4% HSA, 4% RSA, or 100% rat serum was 73.4 ± 7.1%, 76.4 ± 1.3% and 69.9 ± 5.5%, respectively (mean ± SE, \(N = 3\)). This confirms that plasma protein binding of M6G or morphine in rats is minimal (6) and is similar to humans (20). Therefore, the reduced BBB permeability of M6G, compared with morphine, is not caused by plasma protein binding. Instead, the 57-fold difference between the BBB permeability to M6G and morphine in vivo parallels the 187-fold higher \(P\) for morphine than M6G. As shown in table 1, the brain uptake of M6G is only 1/30th that of morphine after an intravenous dose. The comparable BBB permeability for morphine and M6G recorded by microdialysis fibers (4–6) has recently been clarified (21). Owing to the BBB disruption caused by the insertion of microdialysis fibers, the BBB permeability to poorly diffusible compounds such as sucrose is markedly overestimated with the technique (21). Because the \(P\) and the BBB PS product for sucrose (15) and M6G (this study) are comparable (log \(P\) = −3.40 vs. −2.95; BBB PS 0.390 vs. 0.285), the BBB permeability for M6G is likely to be overestimated by the microdialysis methods as well. With respect to the arterial-venous sampling study in pigs (7), the 50% extraction of both morphine and M6G by the brain is clearly artificial, because the unidirectional extraction of morphine by the brain is <2% (22), and the net extraction cannot exceed the unidirectional extraction. The high extraction values for both morphine and M6G recorded for porcine brain are most likely the result of extracerebral contamination of the central venous effluent, a common problem in arteriovenous sampling methodology (23).

\section*{Analgesia Testing}

Given the low level of brain uptake of M6G, compared with morphine (table 1), it is possible that the analgesia caused by peripheral administration of M6G is not centrally mediated. To address this possibility, we examined the antagonistic effects of naloxone, which crosses the BBB, and the quaternary ammonium analog, naloxone methiodide, which does not enter the brain from blood (11). As shown in fig. 2, the baseline tail-flick latency was 3–4 sec. Administration of M6G by subcutaneous injection resulted in an increase in latency and reached the cut-off time 30 min postdose. Analgesia was completely blocked by naloxone HCl, whereas equal doses of naloxone methiodide showed no effect, compared with the naloxone group. These results suggest that the site of M6G analgesia is in the CNS rather than in the periphery.

In summary, data presented herein show that the BBB permeability to M6G is markedly reduced, compared with morphine after intravenous administration to the rat, which corresponds to a 187-fold lower \(P\) for M6G relative to morphine. Despite the poor brain penetration of M6G, the peripheral administration of the compound causes a CNS-mediated analgesia (fig. 2) comparable with morphine (3). Because M6G has been consistently shown to have either comparative or several-fold lower affinities for the total \(\mu\), \(\delta\), or \(\kappa\)-receptors relative to those of morphine (2, 24, 25), these combined results in the present studies provide indirect support to the notion that M6G may activate a novel \(\mu\)-receptor subtype in the brain as suggested by Rossi et al. (26).

\begin{table}[h]
\centering
\caption{Comparison of pharmacokinetics, brain uptake parameters, and lipid solubility for morphine and M6G.}
\begin{tabular}{|l|c|c|}
\hline
Parameter & \(^{[3}H\)Morphine & \(^{[3}H\)M6G \\
\hline
Pharmacokinetics & & \\
\(A_1\) (%ID/ml) & 1.66 ± 0.12 & 3.24 ± 0.43 \\
\(A_2\) (%ID/ml) & 0.228 ± 0.014 & 0.408 ± 0.084 \\
\(K_1\) (min\(^{-1}\)) & 0.868 ± 0.058 & 0.596 ± 0.001 \\
\(K_2\) (min\(^{-1}\)) & 0.0189 ± 0.0013 & 0.0256 ± 0.0090 \\
\(t_{1/2}\) (min) & 37 ± 3 & 36 ± 13 \\
\(t_{10^2}\) (min) & 10.1 ± 0.4 & 18.2 ± 0.9 \\
\(AUC_{0-60}\) (%ID/min/ml) & 14.0 ± 0.7 & 24.4 ± 4.7 \\
\(AUC_{0-\infty}\) (%ID/min/ml) & 8.018 ± 0.0005 & 0.00256 ± 0.00038 \\
\(V_c\) (ml/kg) & 220 ± 13 & 85 ± 10 \\
\(V_s\) (ml/kg) & 1355 ± 73 & 453 ± 88 \\
\(CL\) (ml/min/kg) & 29.5 ± 1.8 & 13.3 ± 2.5 \\
MRT (min) & 46 ± 4 & 40 ± 16 \\
\hline
Brain uptake & & \\
\(V_d\) (ml/g) & 1005 ± 64 & 45 ± 19 \\
PS (ml/min/g) & 8.03 ± 0.28 & 0.142 ± 0.026 \\
% ID/g & 0.0810 ± 0.0005 & 0.00256 ± 0.00038 \\
Lipid solubility & & \\
\(P\) of 1-octanol/buffer & 0.207 ± 0.002 & 0.00111 ± 0.00007 \\
\hline
\end{tabular}
\end{table}

\begin{figure}[ht]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Time course of M6G-induced analgesia and antagonism by naloxone HCl (NLX) and naloxone methiodide (Meth-NLX).}
\end{figure}

Each point represents the mean ± SE of three rats. The dose of M6G, naloxone HCl, and naloxone methiodide is 5.0, 3.7, and 4.7 mg/kg, respectively, which is 10 \(\mu\)mol/kg for all three compounds.

\section*{References}


BRAIN PENETRATION OF MORPHINE-6-GLUCURONIDE


