3H-Morphine-6β-Glucuronide Binding in Brain Membranes and an MOR-1-Transfected Cell Line1

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ABSTRACT

Morphine-6β-glucuronide (M6G) is a potent morphine metabolite. In an effort to further explore its mechanisms of action, we synthesized [3H]-M6G of high specific activity and examined its binding. Although its affinity toward traditional mu receptors is similar to morphine in binding assays in brain and in Chinese hamster ovary cells stably transfected with MOR-1, M6G is >100-fold more potent than morphine in analgesic assays. This apparent discrepancy cannot be explained by differing intrinsic activities of the two drugs because both agents are partial agonists with similar efficacies in adenylyl cyclase assays in the transfected cell lines. Behavioral studies have implied the possibility of a distinct M6G receptor. Detailed binding studies in brain tissue reveal evidence for heterogeneity. Nonlinear regression analysis of 3H-M6G saturation studies reveals two components. The lower-affinity component (K_D = 1.93 ± 0.6 nM) corresponds to labeling of traditional mu receptors. In addition, 3H-M6G labels another site of low abundance with very high affinity (K_D = 68 ± 7 pM). Competition studies indicate that both sites are relatively mu selective. However, several compounds clearly distinguish between the two sites. These binding studies support the concept of a unique M6G receptor responsible for its analgesic activity.

The pharmacology of morphine has proved to be quite complex, due in large part to the activity of a number of important metabolites, including M6G. Although demonstrated to be active >20 years ago (Shimomura et al., 1971; Yoshimura et al., 1973), the importance of M6G was not appreciated until recently (Abbott and Palmour, 1988; Benyhe, 1994; Osborne et al., 1990; Pasternak et al., 1987; Paul et al., 1989; Sullivan et al., 1989; Tiseo et al., 1995). M6G is >100-fold more potent than morphine when administered centrally (Pasternak et al., 1987; Paul et al., 1989). However, this extraordinary activity compared with morphine is not supported by comparisons between the two agents in traditional binding assays, in which morphine is actually slightly more potent than M6G (Pasternak et al., 1987; Paul et al., 1989). This apparent discrepancy between the behavioral and binding studies has raised questions as to the mechanisms of action for M6G analgesia and the possibility of a unique M6G receptor.

Antisense studies have proved to be valuable in the evaluation of opioid analgesia (Pasternak and Standifer, 1995). Initial studies found that antisense oligodeoxynucleotide probes targeting the cloned mu, delta and kappa-1 receptors display selectivities exceeding those of traditional antagonists (Chien et al., 1994; Pan et al., 1994; Rossi et al., 1994; Standifer et al., 1994b), results that have been extended (Adams et al., 1994; Bilsky et al., 1994, 1996; Chen et al., 1995; Chien and Pasternak, 1995; Kolesnikov et al., 1996; Lai et al., 1994, 1995, 1996; Leventhal et al., 1996; Pan et al., 1995; Rossi et al., 1995a, 1995b, 1997; Tseng et al., 1994). In antisense mapping studies (Rossi et al., 1995b, 1995a, 1997), oligodeoxynucleotide probes targeting the exons comprising MOR-1 reveal very different selectivities for morphine and M6G analgesia. Four separate probes targeting exons 1 and 4, which block morphine analgesia in the mouse, are inactive against M6G analgesia. Conversely, five antisense oligodeoxynucleotides based on exons 2 and 3, which are inactive against morphine, effectively block M6G analgesia. Although the reasons for these differences are still unclear, these studies raise the possibility that M6G and morphine analgesia may be mediated through distinct receptors, possibly splice variants of MOR-1 (Pasternak and Standifer, 1995; Rossi et al., 1995a, 1995b, 1997). In an effort to determine the recep-

ABBREVIATIONS: TAPP, Tyr-o-Ala-Phe-Phe-NH₂; PL-017, Tyr-Pro-N-Me-Phe-o-Pro-NH₂; M6G, morphine-6β-glucuronide; DADLE, [o-Ala², o-Leu⁵]enkephalin; DAMGO, [o-Ala², Me-Phe⁶,Gly⁷(ol)]enkephalin; CHO, Chinese hamster ovary; PBS, phosphate-buffered saline.

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tor systems responsible for M6G analgesia, we recently synthesized $^3$H-M6G at very high specific activity (Ouerfelli et al., 1997). We now describe the binding of $^3$H-M6G in brain, membranes and CHO cells stably transfected with MOR-1.

Materials and Methods

Materials. $^3$H-DAMGO and $^3$H-naloxone were obtained from New England Nuclear (Boston, MA). $^3$H-Diprenorphine was purchased from Amersham Life Sciences Inc. (Arlington Heights, IL). $^3$H-M6G was synthesized as previously reported (Ouerfelli et al., 1997). All unlabeled opioids and opioid peptides were the generous gift of the Research Technology division of NIDA. Unless specified, all other chemicals were purchased from Fisher Scientific (Pittsburgh, PA).

Transfection and cell culture. CHO.K1 cells (American Type Culture Collection, Rockville, MD) were maintained in tissue culture flasks in F-12 media supplemented with 10% heat-inactivated fetal bovine serum (Atlanta Biologicals, Atlanta, GA). Cells were grown in a 6% CO$_2$/94% air humidified atmosphere at 37°C. Plates of cells were used at 75% to 95% confluence. Cells were lifted from the substrate for assay or subculturing after a 5-min incubation at 37°C in 5 ml of PBS containing trypsin.

Cells were transfected with DNA (20 μg) encoding the cloned mu opioid receptor MOR-1 cloned into the HindIII site of pRCMV (a generous gift from Dr. L. Yu) or vector without insert by precipitation onto CHO.K1 cells 50% to 60% confluent using DEAE-Dextran (1 mg/ml) and chloroquine (0.1 mM) in normal culture media. After a 3.5-h incubation at 37°C, the transfection media was removed, the cells were washed thrice with PBS and normal culture media was added back to the cells. At 72 hr later, the cells were trypsinized and replanted in selection media (F-12/10% fetal bovine serum/0.4 mg/ml G418; GibCO, Gaithersburg, MD). Individual colonies were cloned and screened for their ability to bind the nonselective opioid antagonist $^3$H-diprenorphine (0.5 nM). After selection, the concentration of G418 was reduced to 0.2 mg/ml in the culture medium.

Receptor binding assays. As previously reported (Clark et al., 1989, 1988), brain tissue was homogenized in 50 volumes of Tris buffer (50 mM Tris, pH 7.7, at 25°C) containing phenylmethylsulfonyl fluoride (0.1 mM) and sodium EDTA (1 mM) in normal culture media. After a 3.5-h incubation at 37°C, the homogenate was removed, the tissue prepared in this manner and kept frozen at −70°C retained its binding for ≥6 weeks.

All $^3$H-DAMGO, $^3$H-M6G and $^3$H-morphine binding assays were performed in 1- or 2-ml volumes (50 mM K$_2$PO$_4$ buffer, pH 7.2) at 25°C for 150 min, and samples were centrifuged for 10 min at 37°C in 5 ml of PBS containing trypsin.

Association of $^3$H-M6G binding to calf striatal membranes. The samples were centrifuged for 10 min at 1000 x g, and the supernatant was assayed for cAMP levels by displacement of $^3$H-cAMP binding to bovine adrenal cortex extract (Sigma Chemical, St. Louis, MO) (Cheng et al., 1995; Standifer et al., 1994a). cAMP levels were calculated from a standard curve determined with unlabeled cAMP.

Statistical analysis. Nonlinear regression analyses of binding and adenylyl cyclase data were performed using the program Prism (GraphPAD Software, San Diego, CA), and a two-site model was adopted at the level of P < .05.

Results

Binding conditions. First, we optimized $^3$H-M6G binding conditions. Binding is rapid at 25°C, reaching steady state levels within 90 min (fig. 1). Binding is linear with increasing amounts of tissue up to 15 mg of wet wt. tissue/ml (fig. 2). We also explored the buffer conditions and ion sensitivity of $^3$H-M6G binding (fig. 3). In the absence of added ions, increasing the ionic strength of the potassium phosphate buffer from 10 to 50 mM increases binding. Magnesium ions enhance binding at the lower buffer concentration, but they have little effect at the higher one. The chelating agent EDTA markedly diminishes the binding of $^3$H-M6G. Sodium chloride also lowers binding, as previously reported for other radiolabeled opioid agonists (Pert et al., 1973; Snyder et al., 1974), but its actions are not as dramatic as those of EDTA. $^3$H-M6G binding is quite sensitive to guanyl-5′-yl-imidodiphosphate (100 μM), which lowered specific binding by ~90% (data not shown).

$^3$H-M6G binding and adenylyl cyclase effects in transfected cells. We next examined $^3$H-M6G binding in CHO cells stably transfected with the MOR-1 clone, as described in Materials and Methods. Saturation analysis revealed that $^3$H-M6G binds to membranes from this transfected cell line with a $K_D$ value of 3.3 nM (table 1). Nonlinear regression analysis of the saturation results implies a single site, and the Scatchard plot is linear (data not shown). The $K_D$ values for the other radiolabeled opioids are similar to

![Fig. 1. Association of $^3$H-M6G binding to calf striatal membranes. Total and nonspecific $^3$H-M6G binding (0.2 nM) was determined at 25°C at the stated times in a 1-ml volume. Nonspecific binding did not change over the time period examined. Specific binding was determined as the difference between total and nonspecific binding. Nonlinear regression using the program Prism shows that $^3$H-M6G associates with an observed rate $k_2$ of 25.6 ± 5.4 min. Results are presented as the mean specific binding ± S.E.M. of three independent determinations.](image-url)
TABLE 1

<table>
<thead>
<tr>
<th>Radioligand</th>
<th>$K_D$ (nM)</th>
<th>$B_{max}$ (fmol/mg)</th>
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<tbody>
<tr>
<td>NaCl (100 mM)</td>
<td>3.32 ± 1.1</td>
<td>190 ± 11</td>
</tr>
<tr>
<td>MgSO$_4$ (5 mM)</td>
<td>1.17 ± 0.26</td>
<td>306 ± 41</td>
</tr>
<tr>
<td>EDTA (5 mM)</td>
<td>2.76 ± 0.5</td>
<td>446 ± 44</td>
</tr>
<tr>
<td>NaI</td>
<td>0.46 ± 0.02</td>
<td>406 ± 19</td>
</tr>
<tr>
<td>NaI</td>
<td>0.49 ± 0.2</td>
<td>526 ± 48</td>
</tr>
</tbody>
</table>

NaBzoH, (-)-6-deoxy-6-benzoylhydrazido-N-allyl-14-hydroxydihydromorphinone.

Fig. 2. Tissue linearity of $^3$H-M6G binding. Calf striatal membranes at the stated protein concentrations were incubated with $^3$H-M6G (0.2 nM) in a 2-ml volume as described in Materials and Methods. Results are presented as the mean ± S.E.M. of three independent determinations.

Fig. 3. Ion sensitivity of $^3$H-M6G binding. Calf striatal membranes were incubated with $^3$H-M6G (0.2 nM) in the presence of MgSO$_4$ (5 mM) or NaCl (100 mM) a 2-ml volume of 10 or 50 mM potassium phosphate buffer, pH 7.2. Specific binding was then determined and compared with controls assayed in the absence of added cations. Results are presented as the mean ± S.E.M. of three independent determinations.

Major differences in efficacy might explain the apparent discrepancy between the relative potencies of morphine M6G in binding and behavioral studies. To explore this possibility further, we examined the functional activity of morphine, M6G and several other opioids on the adenylyl cyclase system in the stably transfected MOR-1-transfected cell line (table 4). As expected, the potencies of the various compounds vary, ranging from an IC$_{50}$ value of 1.2 nM for etorphine to 1.3 µM for morphine. However, the efficacy of a compound in this system is defined by its maximum inhibition. Etorphine, DADLE or DAMGO all inhibit forskolin-stimulated adenylyl cyclase equally well (by ~80%). In contrast, the maximal responses for morphine and M6G are both significantly less than those for the other three agents (P < .05) and are not different from each other. Although M6G is slightly more potent than morphine, their similar maximal responses indicate that efficacy differences cannot explain their markedly different behavioral potencies.

$^3$H-M6G binding in brain. $^3$H-M6G saturation studies in calf striatum reveal curvilinear Scatchard plots (fig. 4) that consist of two binding components based on nonlinear regression analysis of the saturation data. The lower-affinity component ($K_D$ = 1.9 nM) has an affinity similar to that seen in the transfected cell lines ($K_D$ = 3.3 nM). The higher-affinity component ($K_D$ = 68 pM), which represents only ~10% of the $^3$H-M6G sites labeled in calf striatum, is not present in the transfected cell lines.

In the competition studies comparing $^3$H-M6G and $^3$H-morphine binding in the calf striatum, most compounds show similar potencies against both radioligands, with a selectivity suggestive of a $\mu$ receptor (table 3). The $\mu$ compounds such as morphine, DAMGO, codeine, $\delta$-Pen$_2$$\delta$-Pen$_3$enkephalin and the $\kappa$ receptor U50,488H (trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneaceticamide) are both inactive at nanomolar concentrations. Against $^3$H-morphine, all the $\mu$ ligands have Hill coefficients of approximately unity. In contrast, several compounds demonstrate low Hill coefficients against $^3$H-M6G binding. Two peptides, TAPP and PL-017, show Hill coefficients of 0.6 and 0.7, respectively. 3-Methoxyenaxtren shows the most shallow competition curve, with a Hill coefficient of 0.45. Computer analysis of the 3-methoxyenaxtren competition curve is best fit by two components, with IC$_{50}$ values that correspond to $K_D$ values of ~1 and ~135 nM. In contrast, 3-methoxyenaxtren competes $^3$H-morphine binding in brain and $^3$H-M6G binding in the MOR-1-transfected cell line with relatively poor affinity and with a Hill coefficient of approximately unity.

M6G itself also competes $^3$H-M6G binding in brain with a Hill coefficient far less than unity (table 3). The competition curve can be resolved into two components by nonlinear regression analysis, sites that can be observed directly in detailed competition studies (fig. 5). Conversely, the initial inhibition at low M6G concentrations is not present in similar studies with the transfected cell line (fig. 5). The sensitive site in brain, with an IC$_{50}$ value of 0.5 nM, corresponds reasonably well to the high-affinity binding component seen in saturation studies, whereas the less sensitive component in the competition studies (IC$_{50}$ = 19.2 nM) is similar to the
low-affinity site in the saturation studies and corresponds well to the values seen previously in competition studies against traditional \textit{mu} radioligands (Paul et al., 1989). The \textit{IC}_{50} value of M6G against $^3$H-M6G binding in the MOR-1-transfected cell line is similar to the less sensitive component seen in brain competition studies, implying that this component corresponds to a traditional \textit{mu} receptor. The regional distribution of the M6G-sensitive $^3$H-M6G site varies in calf brain (fig. 6). On the basis of detailed competition studies, the levels of high-affinity sensitive M6G binding is \textasciitilde25\% in both striatum and frontal cortex. Slightly lower levels are seen in the brainstem, whereas the periaqueductal gray matter is much lower. It is interesting that the thalamus has no statistically significant high-affinity competition.

**Discussion**

Morphine has an extremely complex pharmacology. Although morphine has been studied for many years, an appreciation of the importance of its active metabolites, particularly M6G, has come only recently. The apparent discrepancy between the far greater analgesic potency of M6G compared with morphine and their similar properties in binding stud-

**TABLE 2**

\textit{\textsuperscript{3}H-M6G} and \textit{\textsuperscript{3}H-morphine binding in CHO/MOR-1 membranes}

Using CHO cells stably transfected with MOR-1, competition studies were performed with the indicated compounds against $^3$H-M6G (1 nM) and $^3$H-morphine (1 nM) binding. Results are mean \pm S.E.M. of at least three independent determinations.

<table>
<thead>
<tr>
<th>Opioid</th>
<th>$^3$H-M6G</th>
<th>$^3$H-Morphine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_i$  nM</td>
<td>$n_H$</td>
</tr>
<tr>
<td>Morphine</td>
<td>18.1 \pm 3</td>
<td>0.83 \pm 0.05</td>
</tr>
<tr>
<td>M6G</td>
<td>14.2 \pm 2.6</td>
<td>1.17 \pm 0.12</td>
</tr>
<tr>
<td>DAMGO</td>
<td>0.78 \pm 0.1</td>
<td>0.84 \pm 0.01</td>
</tr>
<tr>
<td>TAPP</td>
<td>2.7 \pm 0.6</td>
<td>0.97 \pm 0.1</td>
</tr>
<tr>
<td>PL-017</td>
<td>8.63 \pm 0.15</td>
<td>0.85 \pm 0.01</td>
</tr>
<tr>
<td>3-Methoxynaltrexone</td>
<td>255 \pm 36</td>
<td>0.93 \pm 0.08</td>
</tr>
</tbody>
</table>

**TABLE 3**

\textit{\textsuperscript{3}H-M6G binding in mouse brain and CHO/MOR-1 membranes}

Competitions were performed against $^3$H-M6G (1 nM) and $^3$H-morphine (1 nM) with the indicated agents. Results are mean \pm S.E.M. of at least three independent determinations.

<table>
<thead>
<tr>
<th>Opioid</th>
<th>$^3$H-M6G</th>
<th>$^3$H-Morphine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$IC_{50}$ nM</td>
<td>$n_H$</td>
</tr>
<tr>
<td>NalBzoH</td>
<td>0.34 \pm 0.12</td>
<td>1.11 \pm 0.1</td>
</tr>
<tr>
<td>Naloxone</td>
<td>9.1 \pm 3.3</td>
<td>0.89 \pm 0.07</td>
</tr>
<tr>
<td>Diprenorphine</td>
<td>0.42 \pm 0.1</td>
<td>1.11 \pm 0.1</td>
</tr>
<tr>
<td>Morphine</td>
<td>10.2 \pm 5.3</td>
<td>0.94 \pm 0.07</td>
</tr>
<tr>
<td>M6G</td>
<td>0.5 \pm 0.1</td>
<td>0.60 \pm 0.1</td>
</tr>
<tr>
<td>6-Acetylmorphine</td>
<td>19.2 \pm 0.5</td>
<td>1.09 \pm 0.04</td>
</tr>
<tr>
<td>Codeine</td>
<td>415 \pm 131</td>
<td>0.82 \pm 0.04</td>
</tr>
<tr>
<td>6-Acetylcodeine</td>
<td>197 \pm 36</td>
<td>0.85 \pm 0.05</td>
</tr>
<tr>
<td>3-Methoxynaltrexone</td>
<td>11.5 \pm 5.2</td>
<td>0.45 \pm 0.08</td>
</tr>
<tr>
<td>DAMGO</td>
<td>413 \pm 80</td>
<td></td>
</tr>
<tr>
<td>CTAP</td>
<td>2.2 \pm 0.44</td>
<td>1.02 \pm 0.03</td>
</tr>
<tr>
<td>USO,48H</td>
<td>8.89 \pm 0.78</td>
<td>0.97 \pm 0.06</td>
</tr>
<tr>
<td>DPDPDE</td>
<td>\textasciitilde1000</td>
<td>\textasciitilde1000</td>
</tr>
<tr>
<td>Naltindol</td>
<td>37.5 \pm 13</td>
<td>1.14 \pm 0.06</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>1.96 \pm 0.44</td>
<td>0.86 \pm 0.06</td>
</tr>
<tr>
<td>TAPP</td>
<td>1.76 \pm 0.26</td>
<td>0.62 \pm 0.06</td>
</tr>
<tr>
<td>PLO17</td>
<td>9.43 \pm 3.3</td>
<td>0.70 \pm 0.07</td>
</tr>
<tr>
<td>Etonitazene</td>
<td>0.165 \pm 0.03</td>
<td>0.85 \pm 0.04</td>
</tr>
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</table>

CTAP, o-Phe-Cys-Tyr-o-Trp-Arg-Thr-o-Pen-Thr-NH\textsubscript{2}; DPDPDE, [o-Pen\textsuperscript{2},\textsuperscript{6}-Pen\textsuperscript{2}]enkephalin.
results are a representative saturation experiment with 3H-M6G (0.04–3.0 nM) in calf striatal homogenates and are presented as the Scatchard plot, which has been replicated three times. In the brain homogenates, binding was best fit to two sites by nonlinear regression analysis of all independent replications. Site 1, \( K_D = 68 \pm 7 \) pM and \( B_{\text{max}} = 6.5 \) fmol/mg of protein. Site 2, \( K_D = 1.93 \pm 0.6 \) nM and \( B_{\text{max}} = 82.8 \pm 8.3 \) fmol/mg of protein.

A number of behavioral studies have suggested a distinct M6G receptor (Rossi et al., 1996a). CXBK mice, which are insensitive to morphine and other traditional mu ligands such as DAMGO, retain their sensitivity toward M6G, and mice tolerant to morphine in a daily-injection paradigm do not demonstrate tolerance to M6G. Furthermore, antisense mapping studies of MOR-1 have provided strong evidence for distinct receptors (Rossi et al., 1995a, 1995b, 1997). Antisense studies are based on the mRNA sequence being targeted, providing a molecular probe of behavior with a selectivity far better than any of the antagonists currently available. Antisense mapping, in which various exons of a given protein are individually targeted, can provide detailed insights into the function of the protein, particularly regarding the possibility of alternative splicing. The MOR-1 antisense mapping studies exploring the analgesic actions of morphine and M6G are quite intriguing. The abilities of some probes to block morphine and not M6G and others to show the opposite specificity strongly argue for differences at the molecular level between the actions of the two drugs and raise the possibility that they represent splice variants of MOR-1. The activity of all the probes in at least one assay system indicates that technical factors cannot explain these differences. Antisense approaches also have been used to explore the role of specific G protein \( \alpha \) subunits in behavioral actions (Raffa et al., 1994; Sánchez-Blázquez et al., 1995). In extensions of this approach, we also have demonstrated differences in the G protein \( \alpha \) subunits mediating morphine and M6G analgesia (Rossi et al., 1996, 1995b; Standifer et al., 1996). More recent work suggests that heroin and its active metabolite 6-acetylmorphine also can act through this M6G receptor (Rossi et al., 1998). However, a more direct demonstration of a M6G site is needed. Our current binding studies support the concept of a unique 3H-M6G binding site.

In the transfected cell line, M6G binding is readily observed and demonstrates an affinity and selectivity similar to those of traditional mu radioligands. The selectivity of 3H-M6G in the cell line is similar to that observed with 3H-morphine, and the affinity of M6G corresponds well to prior values obtained in brain binding studies. However, the results differ dramatically when extended to brain membranes.
Saturation analysis of $^3$H-M6G is best fit by two sites, which is in contrast to binding in the transfected cell line, which has only a single site. The lower-affinity binding component in brain corresponds quite well to the site in the transfected cell line and appears to represent a traditional mu receptor, whereas the high-affinity component appears to be unique. Competition studies indicate that $^3$H-M6G binding in brain shows an overall selectivity most consistent with mu receptors; however, several compounds strongly imply binding heterogeneity in brain. Several compounds, particularly M6G and 3-methoxynaltrexone, have shallow competition curves with Hill coefficients of less than unity against $^3$H-M6G binding in brain homogenates but not in parallel studies in the transfected cell lines, in which their Hill slopes are unity. In addition, these same compounds compete $^3$H-morphine binding in brain with Hill slopes of approximately unity, clearly documenting the differences between $^3$H-M6G and $^3$H-morphine binding in brain. Similarly, the $^3$H-M6G binding in brain is readily distinguished from $^3$H-M6G binding in the transfected cells line.

M6G itself shows a biphasic competition curve in brain homogenates that is easily broken into two components by nonlinear regression analysis. The very sensitive binding component, corresponding to the high-affinity site seen in saturation studies, represents only a small proportion of the $^3$H-M6G binding, whereas the major component of the binding reflects labeling of traditional mu receptors. It is particularly important to note that this high-affinity site is not seen in the transfected cell lines. This biphasic competition by M6G probably does not reflect differences between agonist and antagonist conformations. 3-Methoxynaltrexone is an opioid antagonist and should label both agonist and antagonist conformations of the receptor equally well, yet 3-methoxynaltrexone also competes $^3$H-M6G binding in brain with a shallower slope and can be dissociated into two components by nonlinear regression analysis. Furthermore, 3-methoxynaltrexone competes $^3$H-morphine binding in brain quite poorly, with a Hill slope not significantly different from unity, a result similar to that against $^3$H-M6G binding in the transfected cell line. Thus, 3-methoxynaltrexone identifies a unique site in brain that is labeled by $^3$H-M6G but not by $^3$H-morphine.

The distribution of the high-affinity $^3$H-M6G binding component also varies among brain regions, with the highest levels in the striatum and frontal cortex. The absence of observable high-affinity binding in the thalamus is particularly interesting in view of the relatively high levels of both mu-1 and mu-2 receptors in this region (Clark et al., 1988; Moskowitz and Goodman, 1985). The antisense mapping studies have strongly suggested important differences between the M6G site and the two mu receptor subtypes, and this regional distribution supports their separation. The presence of the high-affinity M6G site in the periaqueductal gray is important because M6G is an exceptionally potent analgesic when microinjected into this region in rats (Pasternak et al., 1987).

In conclusion, our binding studies reveal $^3$H-M6G binding heterogeneity. The saturation and competition studies support the presence of a novel, high-affinity M6G binding site that might be responsible for the potent analgesic actions of M6G in vivo. Characterization of this site is difficult due to its low abundance, and future studies would be greatly facilitated by a selective binding assay. The availability of agents such as 3-methoxynaltrexone that can distinguish between the two sites may prove valuable in this effort. The low abundance of the M6G site also emphasizes the need for radioligands of very high specific activity. Although the evidence for a unique M6G receptor is quite strong, this putative receptor still must be cloned to prove its existence.

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References


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