Chronic morphine administration augments benzodiazepine binding and GABA_A receptor function*

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Abstract. Behavioral and neurochemical evidence indicates links between the opioid and GABA neurotransmitter systems. To assess effects of chronic opiates on the major site of postsynaptic GABAergic activity, the GABA_A receptor, we administered chronic morphine and naltrexone to mice and evaluated binding at the benzodiazepine and t-butylbicyclophosphorothionate (TBPS) sites and GABA-dependent chloride uptake. After morphine (3 days), benzodiazepine receptor binding in vivo but not in vitro was increased in cortex compared to placebo-treated mice. TBPS binding was unchanged in cortex, but muscimol-stimulated chloride uptake was increased at low doses of muscimol. Benzodiazepine and TBPS binding and muscimol-stimulated chloride uptake were unchanged in naltrexone-(8 days) compared to placebo-treated mice. When naltrexone was administered previously to block opiate sites, the increases in benzodiazepine binding and chloride uptake observed with chronic morphine were reversed. These results indicate that chronic morphine but not naltrexone enhances benzodiazepine binding and GABA_A receptor function, perhaps by an action at opioid receptors.

Key words: Morphine – Naltrexone – GABA – Benzodiazepine – Chloride

Several lines of evidence indicate interactions between the opioid and GABA neurotransmitter systems. Clinical studies report a substantial prevalence of benzodiazepine use among opiate abusers (Stitzer et al. 1981; Woods et al. 1987), perhaps indicating a convergence in effects on central reward systems. In animal behavioral studies, antagonists in the two systems have overlapping effects. For example, opiate antagonists block benzodiazepine-induced hyperphagia in rats (Cooper et al. 1983a), while a benzodiazepine antagonist inhibits the respiratory depressant effects of opiates (Naughton et al. 1985). In addition, histochemical studies demonstrate that GABA and opiates co-localize to neurons in several brain regions (Guidotti et al. 1983; McEachern et al. 1985). These results indicate a possible neurochemical interaction between the opiate and GABA systems. In prior studies, benzodiazepine administration has been reported to modulate release of endogenous opiates in several brain regions (Harsing et al. 1982), while opioid administration may alter benzodiazepine binding at the GABA_A receptor complex (Sivam and Ho 1982; Smith et al. 1984). In addition, endogenous peptides with anti-opiate effects also interact with the GABA_A receptor complex (Miller and Kastin 1987; Miller et al. 1987a, b, 1989). Limited information is available concerning the effects of chronic opiates on the GABA system, and in particular at the major site of post-synaptic GABA effect, the GABA_A receptor. To evaluate these effects, we administered chronic morphine and naltrexone to mice and assessed benzodiazepine and chloride channel binding and receptor function in chloride uptake.

Materials and methods

Male CD1 mice, 6–8 weeks of age, were obtained from Charles River (Wilmington, MA). Morphine pellets (75 mg free base, 3 day duration), morphine placebo, naltrexone (30 mg base, 8 day duration) and naltrexone placebo pellets were obtained from NIDA. [3H]Ro15-1788 (specific activity 82 Ci/mmol), [3H]flunitrazepam (specific activity 78 Ci/mmol), [35S]TBPS (specific activity 81 Ci/mmol), and Protosol were obtained from New England Nuclear (Boston, MA). [35S]TBPS and Protosol were obtained from New England Nuclear (Boston, MA).

Drug pellets were inserted subcutaneously under brief ether anesthesia. Naltrexone pellets were cut into quarters prior to implantation (i.e. 7.5 mg naltrexone per mouse). For morphine administration, receptor assays were performed at the end of the drug
release period (3 days). For naltrexone administration, assays were performed at the end of the drug release period (8 days), except in combined morphine/naltrexone studies where assays were performed after 3 days.

**Benzodiazepine receptor binding in vivo.** Binding was determined by specific uptake of \(^{3}H\)Ro15-1788 as described previously (Miller et al. 1987c). Briefly, mice were injected via the tail vein with 3 μCi \(^{3}H\)Ro15-1788 and sacrificed after 20 min. Brains were removed and dissected rapidly, solubilized for 24 h at 40 °C in 2 ml Protosol and counted by scintillation spectrometry. Nonspecific binding was determined by injection of clonazepam, 5 mg/kg, 30 min before radioligand administration.

**Benzodiazepine receptor binding in vitro.** Benzodiazepine receptor binding was performed by the method of Squires and Bresadra (1977). Briefly, synaptosomal membranes (P1) were prepared as described. Mice were decapitated and brains rapidly removed on ice. Brains were dissected and weighed, and then placed in 30 vol 0.32 M sucrose. Tissue was homogenized in a Teflon-glass homogenizer for 20 s and homogenates were centrifuged at 1000 g for 4 °C for 10 min. Pellets were discarded and supernatants were centrifuged at 19000 g for 20 min at 4 °C. Pellets were resuspended with a Polytron (Brinkmann, Lucerne, Switzerland; setting 7, 4 pulses) in 50 vol 50 mM TRIS-HCl (pH 7.4) and centrifuged at 48000 g for 20 min at 4 ° C. Washing was repeated 3 times, and pellets were then resuspended in 50 vol TRIS-HCl as above and frozen at –70 °C at least overnight. Before use, membranes were thawed, resuspended as above and washed once with TRIS-HCl as above. To determine benzodiazepine receptor binding, increasing concentrations of \([35\text{S}]\)TBPS binding. Binding was performed as described previously (Supavilai and Karobath 1983). Membranes were prepared as described above. To a set of duplicate or triplicate tubes, a fixed amount of \([35\text{S}]\)TBPS (2 nM) was added. Varying amounts of unlabeled TBPS (10–150 nM) were added along with 50 mM TRIS-HCl buffer (pH 7.4) to achieve a final volume of 0.5 ml. After incubation for 45 min at 4 °C, 3 ml iced-cold buffer was added to each tube and tubes were filtered on Whatman GF/B filters using a Brandel M30R apparatus (Gaithersburg, MD) and washed twice with buffer. Filters were counted by conventional scintillation spectrometry. Protein was determined according to Simpson and Sonne (1982).

\[^{35}S\text{]}\)TBPS binding. Binding was performed as described previously (Supavilai and Karobath 1983). Membranes were prepared as described above. To a set of duplicate or triplicate tubes, a fixed amount of \[^{35}S\]TBPS (2 nM) was added. Varying amounts of unlabeled TBPS (10–150 nM) were added along with 0.1 ml 2 M NaCl membranes (0.4 ml, approximately 0.1 mg protein) and buffer as above to a final volume of 1 ml. Tubes were incubated in a shaking water bath at 25 °C for 90 min, and incubations were terminated by addition of 3 ml ice-cold buffer and filtration on Whatman GF/B filters as described above. Tubes were washed twice with buffer, and filters counted by conventional scintillation spectrometry. Non-specific binding was determined by the addition of picrotoxin, 10^-5 M.

\[^{36}\text{Cl}^-\) uptake. Chloride uptake was performed using a modification of the method of Schwartz et al. (1986). Briefly, synaptoneurosomes were prepared as described previously. After centrifugation, tissue was resuspended gently in assay buffer (145 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂ and 10 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) with a glass rod and incubated for 15 min at 30 °C. To 200 μl of membranes was added 200 μl of a solution containing \[^{36}\text{Cl}^-\] (0.2 μCi/ml assay buffer) with or without muscimol (final concentration, 1–50 μM). Tubes were vortexed immediately and after 6 s the incubation was terminated by addition of 3 ml ice-cold assay buffer and filtration on Whatman GF/C filters as described above. Filters were counted by conventional scintillation spectrometry. The amount of \[^{36}\text{Cl}^-\] in the absence of muscimol was subtracted from all values to eliminate non-GABA-related uptake.

**Results**

**Benzodiazepine binding**

In vivo. After chronic morphine administration, benzodiazepine receptor binding assessed in vivo was significantly increased in cortex, but not in the other brain regions.

**Table 1. Effects of chronic morphine on benzodiazepine receptor binding and \[^{35}S\]TBPS binding in cortex**

<table>
<thead>
<tr>
<th>BRAIN REGION</th>
<th>K_d (nM)</th>
<th>B_max (pmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>[^{3}H]flunitrazepam</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphine placebo</td>
<td>1.65 ± 0.07</td>
<td>1.03 ± 0.04</td>
</tr>
<tr>
<td>Morphine</td>
<td>1.69 ± 0.15</td>
<td>1.05 ± 0.04</td>
</tr>
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**\[^{3}H\]TBPS**

| Morphine placebo | 19.9 ± 1.2 | 2.34 ± 0.12 |
| Morphine         | 24.8 ± 4.7 | 2.32 ± 0.52 |

Binding was performed in synaptosomal membrane preparations (P₁) from cerebral cortex. Results are mean ± SEM, n = 3 for each group. There are no significant differences.