INTRODUCTION

Opioids play a central role in the control of pain and regulate a number of other behavioural and physiological responses including gastrointestinal mobility, mood and respiration (Bodnar, 2011). Whilst opioid receptors are important targets for pain management, opioid agonists such as morphine are associated with substance abuse and addiction. There is an unmet need to develop novel drugs for the treatment of pain and opioid dependency without the serious problems associated with the clinical use of opioids. Opioids used clinically, e.g. morphine (full agonist), buprenorphine (partial agonist) exert their pharmacological actions via mu (µ)-opioid receptors, while (-)pentazocine (full agonist) exerts its effects via kappa (κ)-opioid receptors (Chen et al., 1991; Richards and Sadée, 1985, Bidlack et al., 2000).

This ex vivo autoradiographic study investigated the occupancy of μ- and κ-opioid receptors in slices of various rat brain regions following peripheral administration of morphine, buprenorphine, and (-)-pentazocine. H[DAMGO] and [HJU-69,593] were used to label μ- and κ-opioid receptors, respectively.

METHODS

1. Male, Sprague Dawley rats (300±50 g) were administered vehicle, morphine (3, 10 and 30), buprenorphine (0.1, 0.3 and 1.0) or (-)pentazocine (5, 10 and 20) and terminated 60 (morphine and buprenorphine) or 30 minutes later (-pentazocine). Doses are kg ip.

2. Brains were removed and postmortem brains were cut, one at the level of the optic chiasm and one at the level of the cerebellum to produce an anterior brain block containing the cortex and striatum and a posterior brain block containing the hippocampus and the peri-aqueductal grey (PAG). Both brain blocks were placed onto cork disks, covered with Tissue Tek™ and rapidly frozen in isopentane.

3. Cortical coronal sections (20 μm) containing the cortex, striatum, hippocampus and peri-aqueductal grey (PAG) were cut. Three adjacent sections were mounted onto each slide. Of these, two sections were used to measure total binding and one section was used to measure non-specific binding.

4. Sections were incubated in 50 mM Tris buffer containing either [H[DAMGO] (2 mM for cortex, striatum and hippocampus or 5 mM for PAG) or [HJU-69,593] (2.5 mM) for 10 or 90 minutes, respectively. Non-specific binding was determined by 50 μM (-)naloxone or 10 μM U-59,593 for [H[DAMGO] or [HJU-69,593 autoradiography, respectively. Binding was terminated by aspiration and sections washed in buffer (3 x 5 minutes), β-emitting tritium radioactivity bound to the sections was rapidly quantified using a Biospace β-Imager (15 slides per run with a 16 h exposure time).

RESULTS

Table 1: ED₅₀ Values for Morphine and Buprenorphine

<table>
<thead>
<tr>
<th>Compound</th>
<th>ED₅₀ Values, mg/kg ip</th>
<th>Value in Brackets Denote Confidence Limits</th>
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<tbody>
<tr>
<td>Morphine</td>
<td>30 mg/kg ip</td>
<td>52 (49%)</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>30 mg/kg ip</td>
<td>10 (8%)</td>
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1. Specific binding of [H[DAMGO] and [HJU-69,593] was high in the cortex, striatum, hippocampus and the PAG in rat brains in the vehicle-treated groups, with low levels of non-specific binding as defined by (-)naloxone or U-59,593, respectively (Figures 1, 2, 3).

2. Morphine (10 and 30 mg/kg ip) significantly occupied μ-opioid receptors labelled by [H[DAMGO] in the rat cortex (49** and 61***), striatum (52** and 74***), and hippocampus (48** and 56**) when compared to the vehicle-treated controls. A significant inhibition of [H[DAMGO] specific binding to μ-opioid receptors was observed in the PAG (33%) following administration of morphine at 30 mg/kg ip (Figure 1). Doses of (-)-pentazocine were used to produce a 50% reduction in binding (ED₅₀) and 95% confidence limits were determined in the cortex, striatum and hippocampus (Table 1). Buprenorphine (0.1, 0.3 and 1.0 mg/kg ip) significantly occupied μ-opioid receptors labelled by [H[DAMGO] in the rat cortex (49**, 92*** and 95***) when compared to the vehicle-treated controls. A significant inhibition of [HJU-69,593] specific binding to μ-opioid receptors was observed in striatum (87** and 89***% and hippocampus (88**, both doses) following administration of buprenorphine at 0.3 and 1.0 mg/kg ip (Figure 2). The ED₅₀ and 95% confidence limits for buprenorphine were determined in the cortex, striatum and hippocampus (Table 1).

3. (-)-Pentazocine (5, 10 and 20 mg/kg ip) significantly occupied κ-opioid receptors labelled by [HJU-69,593 in rat striatum (54**, 44* and 55 **) (Figure 3).

CONCLUSIONS

1. Morphine (μ-opioid agonist) and buprenorphine (partial μ-opioid agonist/κ-opioid antagonist) occupied central μ-opioid receptors in a dose-dependent manner.

2. The ED₅₀ values were similar across the cortex, striatum and hippocampus for morphine and buprenorphine but buprenorphine was more potent at displacing [H[DAMGO] from central μ-opioid receptors in vivo. These data are consistent with the clinical setting where, depending on the formulation, buprenorphine is approximately 25-100 times more potent than morphine as an analgesic (Khanna and Pillarsetti, 2015).

3. All doses of (-)pentazocine (κ-opioid agonist/μ-antagonist) occupied central κ-opioid receptors. There was a clear dose-ceiling effect for (-)pentazocine due to the mixed agonist/antagonist properties of this compound.

4. Using these tool compounds which have a range of opioid receptor profiles, we have validated these ex vivo autoradiography assays for opioid receptors.

5. Using the Biospace β-Imager technique provides a powerful and very rapid (~24hr) ex vivo autoradiography tool to quantify central μ- and κ-opioid receptor occupancy in vivo by novel drug candidates for pain and opioid dependency.

REFERENCES


