Incomplete, Asymmetric, and Route-Dependent Cross-Tolerance between Oxycodone and Morphine in the Dark Agouti Rat

CARSTEN K. NIELSEN, FRASER B. ROSS, and MAREE T. SMITH

School of Pharmacy, The University of Queensland, St. Lucia, Queensland, Australia

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ABSTRACT

Our previous studies indicate that oxycodone is a putative \( \kappa \)-opioid agonist, whereas morphine is a well-documented \( \mu \)-opioid agonist. Because there is limited information regarding the development of tolerance to oxycodone, this study was designed to document the development of tolerance to the antinociceptive effects of chronically infused i.v. oxycodone relative to that for i.v. morphine and 2) quantify the degree of antinociceptive cross-tolerance between morphine and oxycodone in adult male Dark Agouti (DA) rats. Antinociceptive testing was performed using the tail-flick latency test. Complete antinociceptive tolerance was achieved in 48 to 84 h after chronic infusion of equi-antinociceptive doses of i.v. oxycodone (2.5 mg/24 h and 5 mg/24 h) and i.v. morphine (10 mg/24 h and 20 mg/24 h, respectively). Dose-response curves for bolus doses of i.v. and i.c.v. morphine and oxycodone were produced in naive, morphine-tolerant, and oxycodone-tolerant rats. Consistent with our previous findings that oxycodone and morphine produce their intrinsic antinociceptive effects through distinctly different opioid receptor populations, there was no discernible cross-tolerance when i.c.v. oxycodone was given to morphine-tolerant rats. Similarly, only a low degree of cross-tolerance (\( \sim24\% \)) was observed after i.v. oxycodone administration to morphine-tolerant rats. By contrast, both i.v. and i.c.v. morphine showed a high degree of cross-tolerance (\( \sim71\% \) and \( \sim54\% \), respectively) in rats rendered tolerant to oxycodone. Taken together, these findings suggest that, after parenteral but not supraspinal administration, oxycodone is metabolized to a \( \mu \)-opioid agonist metabolite, thereby explaining asymmetric and incomplete cross-tolerance between oxycodone and morphine.

Morphine is recommended by the World Health Organization as the drug of choice for the management of moderate to severe cancer pain (WHO, 1986). Tolerance to the pain-relieving effects of opioids is a problem that may be encountered in a clinical setting in patients receiving these drugs for the relief of chronic pain. Antinociceptive tolerance is well documented in experimental animals such as rats and mice and is characterized by a marked reduction in the pain-relieving effects of an opioid such as morphine after chronic administration. This phenomenon has been shown by numerous laboratories for a range of antinociceptive tests (Milne et al., 1996). Antinociceptive cross-tolerance between opioids is manifested by a significantly reduced antinociceptive effect evoked by a bolus dose of a second opioid in rats rendered tolerant to the first opioid, relative to the antinociceptive effect of the second opioid in opioid-naive rats. Although the development of tolerance to the pain-relieving effects of morphine in rats is well documented, this is not the case for oxycodone.

Oxycodone has been used clinically for over 80 years, but its pharmacology has been studied only relatively recently. The analgesic actions of oxycodone are comparable to those of morphine and are mediated primarily in the central nervous system. Oxycodone has been reported to be \( \sim1.5 \) times more potent than morphine when administered by both the i.v. and oral routes for postoperative and cancer pain relief with fewer side-effects than morphine (Kalso et al., 1991; Heiskanen and Kalso, 1997; Bruera et al., 1998).

The intrinsic antinociceptive effects of oxycodone are mediated by central nervous system opioid receptors, because they are completely attenuated by i.c.v. naloxone, a universal opioid receptor antagonist (Leow and Smith, 1994). Previous studies in our laboratory have shown that the intrinsic antinociceptive effects of oxycodone are mediated by putative \( \kappa \)-opioid receptors (Ross and Smith, 1997) in contrast to mor-
phin (μ-opioid receptor agonist), as oxycodone’s intrinsic antinociceptive effects were markedly attenuated by i.c.v. administration of nornalbuphine (nor-BNI), a selective κ-opioid receptor antagonist but not by the i.c.v. administration of naloxone, a μ1-selective opioid receptor antagonist or naltrindole, a δ-selective opioid receptor antagonist. Importantly, nor-BNI did not attenuate the antinociceptive effects of i.v. morphine in the same dose that completely attenuated the antinociceptive effects of oxycodone and U-69,593 (κ-selective opioid agonist). Because morphine and oxycodone appear to exert their antinociceptive actions via different classes of opioid receptors, this study was designed to quantify the degree of antinociceptive cross-tolerance between morphine and oxycodone in a rat model.

The Dark Agouti (DA) rat was chosen in preference to the Sprague-Dawley rat because the DA rat is genetically deficient in the enzyme (CYP2D1) that catalyzes the O-demethylation of oxycodone to oxymorphine (a potent μ-opioid agonist) (Muralidharan et al., 1989; Cleary et al., 1994; Xu et al., 1995). In this way, the DA rat is a closer model of the human, because O-demethylation of oxycodone to oxymorphone, with subsequent glucuronidation to oxymorphone-3-glucuronide, accounts for less than 5% of an oxycodone dose in humans (Pöyhä et al., 1992; Ross et al., 1993; Kaiko et al., 1996; Lacouture et al., 1996).

Based on our previous findings (Ross and Smith, 1997) that morphine and oxycodone elicit their intrinsic pain-relieving effects through distinctly different populations of opioid receptors (μ and putative κ, respectively), we hypothesized that rats rendered tolerant to morphine would not show cross-tolerance to oxycodone and vice versa. The studies described herein, have been conducted to investigate this hypothesis.

**Experimental Procedures**

**Experimental Animals**

Ethical approval for these studies was obtained from the Animal Experimentation Ethics Committee of The University of Queensland. Adult male DA rats (200 ± 10 g, mean ± S.E.) were purchased from the Australian Animal Resources Center (Perth, Western Australia) and the Central Animal Breeding House, The University of Queensland. Rats were housed in a temperature-controlled room (21 ± 2°C) with a 12-h light/dark cycle, with food and water available ad libitum. Rats were given at least a 4-day acclimatization period before surgery or experimentation.

**Reagents and Materials**

Oxycodone hydrochloride powder was a generous gift from Tasmanian Alkaloids (Westbury, Australia) and morphine sulfate ampules (30 mg/ml) were purchased from the Royal Brisbane Hospital Pharmacy (Brisbane, Australia). Isoflurane (Forthane) was purchased from Abbott Australasia Pty. Ltd. (Sydney, Australia), ketamine hydrochloride vials (100 mg/ml) were purchased from Parnell Laboratories Australia Pty. Ltd. (Sydney, Australia), and xylazine hydrochloride (IIium Xylazil-20) vials (20 mg/ml) were purchased from Troy Laboratories Pty. Ltd. (Sydney, Australia). Normal saline was purchased from Delta West Pty. Ltd. (Perth, Australia) and heparinized saline (50 IU/ml) was purchased from Astra Pharmaceuticals Pty. Ltd. (Sydney, Australia). Single lumen polyethylene tubing (0.5-mm internal diameter) was purchased from Auburn Plastics and Engineering Pty. Ltd. (Sydney, Australia) and Silastic tubing was purchased from Asia Pacific Specialty Chemical Ltd. (Brisbane, Australia). Denture Acrylic and Denture Monomer were purchased from Regional (Brisbane, Australia). Hamilton syringes were used for i.c.v. drug administration, and Graseby medical syringe drivers were used to deliver the i.v. infusions.

**Preparation of Solutions**

Solutions of oxycodone for i.v. infusion were prepared by dissolving oxycodone hydrochloride in normal saline to achieve final concentrations of 0.56 and 0.28 mg/ml. Similarly, solutions of oxycodone for bolus i.v. and i.c.v. administration were prepared to achieve concentrations of 0.16 to 19.03 μmol/ml and 22 to 330 nmol/μl, respectively. Solutions of morphine for i.v. infusion were prepared from morphine sulfate ampules by dilution with normal saline to achieve final concentrations of 2.22 and 1.11 mg/ml. Similarly, solutions of morphine for i.v. and i.c.v. bolus administration were prepared to achieve concentrations of 0.7 to 21.03 μmol/ml and 18 to 225 nmol/μl, respectively. The concentrations of each solution of oxycodone and morphine were verified before dosing using high performance liquid chromatography with electrochemical detection (Wright and Smith, 1998). All solutions for chronic i.v. administration were freshly prepared, and all solutions for i.v. and i.c.v. bolus injection were stored at -20°C until required.

**Implantation of i.c.v. Cannulas**

Deep and stable anesthesia was produced in rats after i.p. administration of a mixture of ketamine (75 mg/kg) and xylazine (5 mg/kg). Stainless steel guide cannulas (8 mm long) were implanted stereotaxically to a depth of 1 mm above the left lateral ventricle according to the rat brain coordinates of Paxinos and Watson (1986): 0.8 mm P, 1.5 mm L and 3.3 mm V from Bregma. Guide cannulas were secured in position with dental cement, and the scalp wound was sutured. Cannula plugs (9 mm long) were kept in place except during i.c.v. drug injections. Following surgery, rats received an i.m. injection of benzylpenicillin (60 mg) to prevent infection. Rats were allowed to recover overnight before jugular vein cannulation was performed.

**Jugular Vein Cannulation**

Polyethylene cannulas (previously filled with heparinized saline) were inserted into the right common jugular vein while rats were under 3% isoflurane:97% oxygen inhalational anesthesia. Jugular vein cannulas were tested for correct placement by withdrawal of a small amount of blood, after which the blood was pushed back into the vein using heparinized saline. Cannulas were exteriorized by tunneling to a s.c. pocket located between the scapulae at the base of the neck. Incisions were closed with sterile silk sutures. After surgery, rats were housed singly in metabolic cages and administered an i.v. infusion of saline overnight, before experimentation the following day. Food and water were available ad libitum during the overnight recovery period and for the duration of the experiment.

**Opioid Dosing Regimes**

**Antinociceptive Tolerance to Oxycodone and Morphine.** Groups of DA rats received i.v. oxycodone at an infusion rate of 2.5 mg/24 h (n = 8) or 5 mg/24 h (n = 8). Additional groups of DA rats received i.v. morphine at an infusion rate of 10 mg/24 h (n = 8) or 20 mg/24 h (n = 8). Infusions were continued until rats were completely tolerant to the antinociceptive effects of the administered opioid (baseline levels of antinociception).

**Cross-Tolerance between Oxycodone and Morphine.** The degree of cross-tolerance between oxycodone and morphine was quantified in the DA rat. Once rats were tolerant to the antinociceptive effects (48–84 h) of the first opioid (i.v. oxycodone 2.5 or 5 mg/24 h or i.v. morphine 10 or 20 mg/24 h), they received a 12-h infusion of saline to ensure clearance of the first opioid and its metabolites. Rats then received either a chronic i.v. infusion of the second opioid in one of the above-mentioned doses (Table 1) or bolus opioid administration by the i.v. (0.5 ml) or the i.c.v. (1 μl) route (Tables 1 and 2).

Groups of naive DA rats also received bolus doses of one opioid
Antinociceptive Testing

The tail-flick latency test was used to quantify antinociception (D'Amour and Smith, 1941), with the thermal stimulus being applied to the lower third of the dorsal surface of the rat's tail. Before drug administration, baseline antinociceptive testing was performed at 5-min intervals until three baseline latency values were within ±1 s (only three readings were required in most rats and no more than five readings were required in any rats). The mean baseline tail-flick latency was 2.5 s, and the range was 1.5 to 4 s. For the chronic infusion studies, antinociceptive testing was performed at the following times after initiation of the i.v. infusion: 0.5, 1, 2, 4, 6, 8, and 12 h, and then every 12 h until the rats were tolerant (baseline levels of antinociception achieved). For the bolus studies, antinociceptive testing was performed at the following postdosing times over a 3-h experimental period: 5, 10, 15, 30, 45, 60, 75, 90, 120, 150, and 180.

TABLE 1
Chronic and bolus i.v. doses of oxycodone, morphine, or saline (control rats) administered to naive, oxycodone-tolerant, and morphine-tolerant adult male DA rats

<table>
<thead>
<tr>
<th>Treatment Period 1: Naive Rats</th>
<th>Treatment Period 2: Tolerant Rats</th>
<th>Treatment Period 3: Tolerant Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxycodeone i.v. infusion, 2.5 mg/24 h for 60 h</td>
<td>i.v. bolus dose of oxycodone</td>
<td>i.v. bolus dose of oxycodone</td>
</tr>
<tr>
<td>20 mg/24 h i.v. oxycodone (n = 4)</td>
<td>1,402 nmol (n = 4)</td>
<td>2,220 nmol (n = 4)</td>
</tr>
<tr>
<td>20 mg/24 h i.v. morphine (n = 8)</td>
<td>2,803 nmol (n = 4)</td>
<td>3,171 nmol (n = 6)</td>
</tr>
<tr>
<td>5 mg/24 h i.v. oxycodone (n = 4)</td>
<td>3,504 nmol (n = 7)</td>
<td>3,805 nmol (n = 4)</td>
</tr>
<tr>
<td>5 mg/24 h i.v. oxycodone (n = 4)</td>
<td>4,205 nmol (n = 4)</td>
<td>4,756 nmol (n = 4)</td>
</tr>
<tr>
<td>20 mg/24 h i.v. morphine (n = 4)</td>
<td>7,098 nmol (n = 4)</td>
<td>9,513 nmol (n = 4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Morphine i.v. infusion, 10 mg/24 h for 60 h</th>
<th>i.v. bolus dose of morphine</th>
<th>i.v. bolus dose of morphine</th>
</tr>
</thead>
<tbody>
<tr>
<td>317 nmol (n = 4)</td>
<td>3,504 nmol (n = 4)</td>
<td></td>
</tr>
<tr>
<td>951 nmol (n = 4)</td>
<td>4,205 nmol (n = 6)</td>
<td></td>
</tr>
<tr>
<td>1,585 nmol (n = 8)</td>
<td>4,906 nmol (n = 4)</td>
<td></td>
</tr>
<tr>
<td>2,537 nmol (n = 8)</td>
<td>6,308 nmol (n = 6)</td>
<td></td>
</tr>
<tr>
<td>6,342 nmol (n = 4)</td>
<td>10,514 nmol (n = 4)</td>
<td></td>
</tr>
</tbody>
</table>

* Each treatment period was followed by a 12-h washout period with saline.

TABLE 2
i.c.v. bolus doses of oxycodone, morphine, or saline (control rats) administered to naive, oxycodone-tolerant, and morphine-tolerant adult male DA rats

<table>
<thead>
<tr>
<th>Treatment Period 1: Naive Rats</th>
<th>Treatment Period 2: Tolerant Rats</th>
<th>Treatment Period 3: Tolerant Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxycodeone i.v. infusion 2.5 mg/24 h for 60 h</td>
<td>i.c.v. bolus dose of oxycodone</td>
<td>i.c.v. bolus dose of oxycodone</td>
</tr>
<tr>
<td>18 nmol (n = 5)</td>
<td>44 nmol (n = 5)</td>
<td></td>
</tr>
<tr>
<td>34 nmol (n = 5)</td>
<td>78 nmol (n = 5)</td>
<td></td>
</tr>
<tr>
<td>55 nmol (n = 5)</td>
<td>132 nmol (n = 5)</td>
<td></td>
</tr>
<tr>
<td>150 nmol (n = 5)</td>
<td>300 nmol (n = 5)</td>
<td></td>
</tr>
<tr>
<td>Morphine i.v. infusion 10 mg/24 h for 60 h</td>
<td>i.c.v. bolus dose of morphine</td>
<td>i.c.v. bolus dose of morphine</td>
</tr>
<tr>
<td>44 nmol (n = 5)</td>
<td>18 nmol (n = 5)</td>
<td></td>
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<td>150 nmol (n = 5)</td>
<td></td>
</tr>
</tbody>
</table>

* Each treatment period was followed by a 12-h washout period with saline.

(oxycodeone or morphine) or saline (control rats) by the i.v. or i.c.v. route (Tables 1 and 2).
A maximum tail-flick latency of 9.0 s was used to minimize tissue damage to the rats’ tails. The tail-flick latency values were converted to a percentage of the maximum possible effect (%MPE) (Brady and Holtzmann, 1982):

\[
%MPE = \frac{\text{Postdrug latency } - \text{Predrug latency}}{\text{Maximum latency (9.0 s) } - \text{Predrug latency}} \times 100
\]

### Data Analyses

Levels of antinociception (%MPE values) were plotted against time to produce response (%MPE versus time curves). Dose-response curves were then produced based on the area under the %MPE versus time curves (%MPE AUC values) for each dose of oxycodone and morphine administered by the i.v. and i.c.v. routes. ED₅₀ doses (the dose required to produce a half-maximal antinociceptive effect) were then estimated using the GraphPad Prism program using a sigmoidal curve with variable slope. %MPE AUC and ED₅₀ values were analyzed for statistical significance using the Mann-Whitney U test as implemented in the Minitab statistical analysis package, with statistical significance criterion of \( P < .05 \).

The degree of cross-tolerance between oxycodone and morphine was calculated using the ED₅₀ doses and expressed as the degree of cross-tolerance (%CT), as shown below for opioid A administration to opioid B-tolerant rats:

\[
%CT = \frac{\text{ED}_{50} \text{ opioid B-tolerant } - \text{ED}_{50} \text{ opioid A-naive}}{\text{ED}_{50} \text{ opioid A-tolerant } - \text{ED}_{50} \text{ opioid A-naive}} \times 100
\]

### Results

After chronic i.v. infusion of oxycodone or morphine in doses of 2.5 mg/24 h and 10 mg/24 h, respectively, to opioid-naive DA rats, complete antinociceptive tolerance was produced by 48 h (Fig. 1). As expected (Ling et al., 1989; Smith and Smith, 1995), a doubling of the opioid infusion rate for each of oxycodone and morphine (5 mg/24 h and 20 mg/24 h, respectively) resulted in a longer time (72–84 h) for the establishment of antinociceptive tolerance (Fig. 1). Chronic i.v. infusion of morphine and oxycodone in a crossover design showed that rats administered i.v. oxycodone in a dose of 5 mg/24 h (Fig. 2A) became completely tolerant after 72, 48, and 8 h for naive, morphine-tolerant, and oxycodone-tolerant rats, respectively. Similarly, rats administered i.v. morphine in a dose of 20 mg/24 h (Fig. 2B) became completely tolerant after 84, 36, and 12 h for naive, oxycodone-tolerant, and morphine-tolerant rats, respectively.

Irrespective of whether naive DA rats were infused with oxycodone (2.5 mg/24 h) or morphine (10 mg/24 h), they achieved significant initial antinociception (≈50% MPE). Additionally, the extent and duration of the antinociceptive effects (area under the degree of antinociceptive effects (area under the degree of antinociceptive versus time curves; %MPE AUC values) produced by chronic infusion of i.v. oxycodone in doses of 2.5 and 5.0 mg/24 h (1165 ± 131% MPE.h and 3737 ± 456% MPE.h, respectively) were not significantly different (\( P > .05 \)) from the corresponding %MPE AUC values produced by chronic i.v. infusion of morphine in doses of 10 and 20 mg/24 h (1239 ± 171% MPE.h and 4876 ± 448% MPE.h, respectively, Fig. 1). Importantly, these findings indicate that equipotent antinociception was produced by chronic infusion of i.v. oxycodone and morphine in doses of 2.5 mg/24 h and 10 mg/24 h, respectively (Fig. 1), and that tolerance was established over a similar time frame (48 h) for each of these opioid infusion regimens.

It was also noted that chronic infusion of oxycodone and morphine in doses of 2.5 mg/24 h and 10 mg/24 h, respectively, resulted in a reduced incidence of side-effects (respiratory depression, marked sedation, and/or central nervous system excitation) compared with the higher opioid infusion rates (5 mg/24 h and 20 mg/24 h for oxycodone and morphine, respectively). Thus, based on these aforementioned observations, our subsequent studies involving the acute i.c.v. or i.v. administration of oxycodone or morphine to opioid-tolerant rats were undertaken in rats that had been made tolerant to...
the antinociceptive effects of oxycodone or morphine in doses of 2.5 mg/24 h and 10 mg/24 h, respectively.

After bolus i.c.v. oxycodone dosing, rats exhibited a single phase of antinociception as expected from previous studies in our laboratory (Leow and Smith, 1994; Ross and Smith, 1997) with a peak antinociceptive response observed 10 to 15 min post drug administration (Fig. 4A). For naive and morphine-tolerant rats, visual inspection of the data revealed that the dose-response curves for i.c.v. oxycodone were clearly indistinguishable (Fig. 3A). Consistent with these observations, the respective ED50 values were not significantly different (P > .05) (Table 3) indicating the absence of supraspinal cross-tolerance after administration of i.c.v. oxycodone to morphine-tolerant rats.

After i.c.v. administration of morphine (55 nmol; Fig. 4C) to naive rats, the antinociceptive response comprised two distinct phases as expected based on our previous studies (Smith et al., 1990; Leow and Smith, 1994; Ross and Smith, 1997). The first phase of antinociception (thought to be due primarily to supraspinal mechanisms) commenced at approximately 15 min, peaked at 45 to 60 min, and decreased at 75 min. The second phase of antinociception (involving spinal antinociceptive activity) peaked at 90 min and decreased throughout the remainder of the 3-h observation period. In both oxycodone-tolerant and morphine-tolerant rats, there was a significant rightward shift in the respective dose-response curves (Fig. 3C), such that the ED50 values for i.c.v. morphine in oxycodone- and morphine-tolerant rats were

Fig. 3. Mean (±S.E.) dose-response curves (area under the degree of tail-flick antinociception versus time curves, versus opioid dose) for naive, morphine-tolerant, and oxycodone-tolerant adult male DA rats that received a bolus dose of i.c.v. oxycodone (A), i.v. oxycodone (B), i.c.v. morphine (C), i.v. morphine (D), i.c.v. morphine (phase 1 antinociception: 0–75 min; E), and i.c.v. morphine (phase 2 antinociception: 75–180 min; F).
antinociception observed in control rats that received either a bolus or i.v. morphine were sedated. Importantly, levels of antinociception (%MPE AUC value) for phase 2 antinociception is significantly lower (P < .05) in tolerant compared with naive rats (Fig. 3F). On this basis, cross-tolerance between i.c.v. morphine and parenteral oxycodone or morphine may be attributable primarily to the marked decrease in the phase 2 antinociceptive effects.

Behaviorally, rats that received either i.c.v. morphine or i.v. oxycodone were sedated. However, only rats that received i.c.v. morphine experienced urinary incontinence, characterized by continuous “dribbling” from the bladder, during the second phase of antinociception.

For i.v. oxycodone, rats exhibited a single-phased antinociceptive response with a peak effect 5- to 10-min post drug administration, which decreased throughout the remainder of the observation period (Fig. 4B). Similarly, for i.v. morphine, rats exhibited a single phase of antinociception with a peak effect at 15- to 30-min post drug administration, which declined thereafter (Fig. 4D). The (mean ± S.E.) ED_{50} doses for i.v. oxycodone and i.v. morphine were significantly different within each set of dose-response curves (P < .05), indicating that there is incomplete cross-tolerance between oxycodone and morphine. Specifically, when i.v. oxycodone was administered to morphine-tolerant rats, there was a significantly (P < .05) lower degree of cross-tolerance (23.7%) than for i.v. morphine administration to oxycodone-tolerant rats (71.3%) (Table 4).

Behaviorally, rats administered bolus doses of i.v. oxycodone or i.v. morphine were sedated. Importantly, levels of antinociception observed in control rats that received either a bolus dose of i.c.v. or i.v. saline did not differ significantly from predose baseline levels of antinociception (P > .05) (Fig. 4, A–D). Additionally, control rats dosed with saline were behaviorally indistinguishable from rats that received no treatments.

Discussion

After chronic infusion of i.v. oxycodone in doses of 2.5 and 5.0 mg/24 h, complete antinociceptive tolerance was produced in 48 and 72 h, respectively. Similarly, a doubling of the rate of chronic i.v. morphine infusion increased the time required for the development of antinociceptive tolerance from 48 to 84 h, consistent with previous studies whereby the time required for the production of antinociceptive tolerance to morphine was directly related to the magnitude of the morphine dose, with larger doses requiring longer times for the development of tolerance (Ling et al., 1989; Smith and Smith, 1995).

Importantly, significant tolerance, manifested by an ~2.6-fold shift in the ED_{50} for i.v. oxycodone, was observed in rats rendered tolerant to i.v. oxycodone. By contrast, there was a total absence of cross-tolerance between supraspinally administered oxycodone and i.v. morphine in the studies described herein. Taken together, these data support previous studies from our laboratory (Ross and Smith, 1997), which showed that the intrinsic antinociceptive effects of oxycodone and morphine are mediated by distinctly different populations of opioid receptors in the rat central nervous system. Additionally, our findings indicate that, when oxycodone is administered supraspinally, i.e., by a route where metabolism is minimal, cross-tolerance with i.v. morphine does not occur, in contrast to the marked tolerance observed when i.c.v. oxycodone is administered to rats rendered tolerant to parenteral oxycodone.

In contrast to the lack of cross-tolerance between i.c.v. oxycodone and i.v. morphine, a relatively high degree of cross-tolerance (54% CT) was observed when i.c.v. morphine was administered to oxycodone-tolerant rats (Table 4). These findings of asymmetric and incomplete cross-tolerance between oxycodone and morphine suggest that after chronic i.v. administration, oxycodone is metabolized to a µ-opioid agonist metabolite, which then produces substantial tolerance to i.c.v. morphine at the µ-opioid receptor. By contrast, after administration of oxycodone by the i.c.v. route where metabolism is negligible, a lack of cross-tolerance would be expected with i.v. morphine, as was observed for i.c.v. oxycodone in the studies described herein.

Intriguingly, when supraspinal morphine was administered to rats rendered tolerant to either i.v. morphine or i.v. oxycodone (Fig. 4C), there appeared to be a differential degree of cross-tolerance between the first and second phases of antinociception relative to naive rats (Fig. 3, E and F). Because the first and second phases of morphine antinociception are thought to be mediated primarily by supraspinal and spinal mechanisms, respectively, our results suggest that antinociceptive tolerance to parenteral morphine may be mediated predominantly at the spinal level. This view is supported by previous findings in mice by Kolesnikov et al. (1996), who reported a much greater degree of tolerance development to daily injections of morphine administered by combined systemic (s.c.) and spinal [intrathecal (i.t.)] routes.
(5–12-fold shift in ED$_{50}$) than when compared with combined injections by the s.c. and i.c.v. routes (2-fold shift in ED$_{50}$). Although mice that received morphine by only the i.c.v. or the i.t. route did not appear to develop significant tolerance in their study (ED$_{50}$ for i.t. morphine shifted by only 1.1-fold), antinociception was quantified at only a single time point (15 min) after drug administration. Thus, if tolerance had occurred to a late component of morphine’s antinociceptive effects in their study, it would not have been detected.

Inspection of the dose-response curves for i.v. oxycodone administered to morphine-tolerant rats (Fig. 3B) or i.v. morphine to oxycodone-tolerant rats (Fig. 3D), reveals that cross-tolerance was far less pronounced when i.v. oxycodone was administered to morphine-tolerant rats than when i.v. morphine was administered to oxycodone-tolerant rats. Specifically, there was a 3.9-fold increase in the ED$_{50}$ for i.v. oxycodone in morphine-tolerant relative to opioid-naive rats, whereas the respective increase in the ED$_{50}$ for i.v. oxycodone administered to oxycodone-tolerant rats was 13.2-fold (Table 3). By contrast, when i.v. morphine was administered to both oxycodone-tolerant and morphine-tolerant rats, the mean ED$_{50}$ values increased to a similar extent (3.2- and 4.1-fold, respectively) relative to that of naive rats (Table 3). Taken together, our findings clearly show incomplete and asymmetric cross-tolerance between parenteral oxycodone and morphine such that the extent of cross-tolerance observed between these two opioids is dependent on the order of drug administration, with the %CT for i.v. morphine administered to oxycodone-tolerant rats (71.3%) being ~3 times greater that for i.v. oxycodone administered to morphine-tolerant rats (23.7%) (Table 4).

Taken together with the supraspinal data, our findings clearly indicate that the receptors mediating the intrinsic antinociceptive effects of oxycodone are distinctly different from those mediating the pain-relieving effects of morphine. Moreover, our current findings provide additional evidence to support our previous work (Ross and Smith, 1997) that showed 1) the intrinsic antinociceptive effects of oxycodone were completely attenuated by the $\kappa$-selective opioid antagonist, nor-BNI, in a dose that did not attenuate the antinociceptive effects of i.c.v. morphine, and 2) the $\mu$-selective opioid antagonist, naloxonazine, did not attenuate the antinociceptive effects of i.c.v. oxycodone in a dose that completely blocked the pain-relieving effects of i.c.v. morphine, indicating that the intrinsic antinociceptive effects of oxycodone are mediated by putative $\kappa$-opioid receptors, whereas morphine is well documented to be a $\mu$-opioid agonist.

Several previous studies have shown an absence and/or asymmetrical cross-tolerance between opioids that bind selectively to different types of opioid receptors. Specifically, an
absence of cross-tolerance has been shown between i.m. morphine (µ-opioid agonist) and i.m. U-50,488H (selective κ-opioid agonist) in pigeons (Craft et al., 1989) and squirrel monkeys (Craft and Dykstra, 1990); between s.c. morphine and s.c. U-50,488H in rats (Picker et al., 1991) and rhesus monkeys (Gmerek et al., 1987), with the latter study also showing a converse lack of cross-tolerance between s.c. U-50,488H and s.c. morphine. A lack of cross-tolerance was also found between morphine (i.m. and s.c.) and the κ-opioid receptor agonists, ethylketazocine (Craft and Dykstra, 1990) and bremazocine (Picker et al., 1991), in squirrel monkeys and rats, respectively.

Our findings of asymmetrical cross-tolerance between oxycodone and morphine are consistent with earlier studies by Gmerek et al. (1987), who reported cross-tolerance between acutely administered s.c. morphine and Mr 2033 (furfuryl-substituted benzomorphan, a κ-opioid receptor agonist) given by chronic s.c. dosing to monkeys, but an absence of cross-tolerance when acute s.c. doses of Mr 2033 were given to morphine-tolerant monkeys. The proposed explanation for the asymmetrical or unidirectional cross-tolerance observed by Gmerek et al. (1987) between Mr 2033 and morphine, was that Mr 2033 was possibly a nonselective κ-opioid receptor agonist. However, another equally plausible explanation is that systemically administered Mr 2033 is metabolized to a µ-opioid receptor agonist metabolite, which then produces cross-tolerance with morphine.

Using similar reasoning, we propose that, after i.v. administration, oxycodone is metabolized to a µ-opioid agonist metabolite, which results in the ≈24% cross-tolerance with parenteral morphine. Despite the clinical use of oxycodone for over 80 years, the major metabolites of oxycodone remain to be elucidated. N-Demethylation and O-demethylation of oxycodone in humans result in the formation of noroxycodone and oxymorphine, respectively (Pöyhia et al., 1991), although these metabolites account for only 15% to 25% and less than 5% of the oxycodone dose, respectively (Ross et al., 1993; Lacouture et al., 1996). Because the DA rats used in the study described herein are genetically deficient in CYP2D1 (enzyme required to O-demethylate oxycodone to oxymorphine), it is doubtful that the µ-opioid, oxymorphine, is responsible for the development of cross-tolerance between morphine and parenteral oxycodone. Metabolism of oxycodone to the N-oxide metabolite or reduction of the 6-ketone group to form the 6α- and 6β-oxycodols also occur, but these are also minor metabolic pathways (Ishida et al., 1982). Up to a further 10% of the oxycodone dose is also eliminated in the urine unchanged. From the foregoing, it is clear that ≈60% of each oxycodone dose remains to be accounted for with the possibility of an as yet unidentified µ-opioid agonist metabolite being formed, which is responsible for the unidirectional cross-tolerance between oxycodone and morphine.

Behaviorally, it was noted that the onset of “dribbling urinary incontinence” after i.c.v. administration of morphine coincided with the onset of phase 2 antinociception, consistent with the caudal spread of relatively high concentrations of morphine to spinal µ-opioid receptors. This observation is consistent with the findings of Igawa et al. (1993) who showed that morphine administered i.t. dose dependently inhibited micturition, resulting in urinary retention and dribbling urinary incontinence. Intriguingly, incontinence was not observed after i.v. morphine dosing in the present study. One possible explanation is that, after parenteral morphine dosing, the cerebral cerebrospinal fluid concentration of morphine is considerably lower than the corresponding cerebrospinal fluid concentration achieved after i.c.v. morphine dosing. Additionally, after parenteral morphine dosing, it is possible that morphine-3-glucuronide, the major metabolite of morphine, prevents the development of dribbling incontinence, because morphine-3-glucuronide given by the i.t. route in rats has been shown to attenuate the morphine-evoked inhibition of the micturition reflex by increasing bladder contractility (Igawa et al., 1993).

In summary, the complete absence of cross-tolerance between supraspinally administered oxycodone and i.v. morphine supports our previously published findings that the intrinsic antinociceptive effects of oxycodone and morphine are mediated through distinctly different populations of opioid receptors (putative κ-opioid receptors and µ-opioid receptors, respectively). Additionally, our findings that both i.c.v. and i.v. morphine show incomplete antinociceptive cross-tolerance with i.v. oxycodone clearly imply that, after parenteral administration, oxycodone is metabolized to a µ-agonist metabolite thereby producing cross-tolerance with subsequently administered morphine.

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Send reprint requests to: A/Prof Maree T. Smith, School of Pharmacy, The University of Queensland, St. Lucia, Queensland 4072, Australia. E-mail: m.smith@pharmacy.uq.edu.au