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RESEARCH PAPER

In vivo profiling of seven common opioids for antinociception, constipation and respiratory depression: no two opioids have the same profile

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BACKGROUND AND PURPOSE

For patients experiencing inadequate analgesia and intolerable opioid-related side effects on one strong opioid analgesic, pain relief with acceptable tolerability is often achieved by rotation to a second strong opioid. These observations suggest subtle pharmacodynamic differences between opioids *in vivo.* This study in rats was designed to assess differences between opioids in their *in vivo* profiles.

EXPERIMENTAL APPROACH

Male Sprague Dawley rats were given single i.c.v. bolus doses of morphine, morphine-6-glucuronide (M6G), fentanyl, oxycodone, buprenorphine, DPDPE ([D-penicillamine^{2,5}]-enkephalin) or U69,593. Antinociception, constipation and respiratory depression were assessed using the warm water tail-flick test, the castor oil-induced diarrhoea test and whole body plethysmography respectively.

KEY RESULTS

These opioid agonists produced dose-dependent antinociception, constipation and respiratory depression. For antinociception, morphine, fentanyl and oxycodone were full agonists, buprenorphine and M6G were partial agonists, whereas DPDPE and U69,593 had low potency. For constipation, M6G, fentanyl and buprenorphine were full agonists, oxycodone was a partial agonist, morphine produced a bell-shaped dose–response curve, whereas DPDPE and U69,593 were inactive. For respiratory depression, morphine, M6G, fentanyl and buprenorphine were full agonists, oxycodone was a partial agonist, whereas DPDPE and U69,593 were inactive. The respiratory depressant effects of fentanyl and oxycodone were of short duration, whereas morphine, M6G and buprenorphine evoked prolonged respiratory depression.

CONCLUSION AND IMPLICATIONS

For the seven opioids we assessed, no two had the same profile for evoking antinociception, constipation and respiratory depression, suggesting that these effects are differentially regulated. Our findings may explain the clinical success of 'opioid rotation'.

LINKED ARTICLES

This article is part of a themed section on Opioids: New Pathways to Functional Selectivity. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2015.172.issue-2

Abbreviations

DPDPE, [D-penicillamine^{2,5}]-enkephalin; GI, gastrointestinal; M6G, morphine-6-glucuronide; MOP, μ-opioid; MPE, maximum possible effect; MV, minute ventilation; NOP, nociceptin opioid; SD, Sprague Dawley; WBP, whole body plethysmography

Introduction

Strong opioid analgesics evoke a range of pharmacodynamic responses in humans and animals, with analgesia being the desired effect (Law *et al.*, 2013). However, in the analgesic dose range, opioid-related adverse effects including nausea, vomiting, sedation, respiratory depression and constipation also commonly occur (Schug *et al.*, 1992; Thorpe, 2001; Koo and Eikermann, 2011). Tolerance develops to some opioid-related adverse effects with chronic dosing but this is not the case for constipation. If not proactively managed, opioid-induced constipation results in significant impairment in the quality of life of patients (Thorpe, 2001). Although tolerance to respiratory depression develops with chronic dosing, its potential for mortality in opioid-naïve patients means that it is the side effect most feared by clinicians in post-surgical settings (Jaffe and Martin, 1991; Hindle, 2008).

Clinically utilized opioid analgesics are generally regarded as μ-opioid (MOP) (Cox *et al.*, 2015) receptor agonists having similar pharmacological profiles (receptor nomenclature follows Alexander *et al.*, 2013). However, clinical experience has shown that for patients experiencing poor analgesia with intolerable opioid-related adverse effects on one strong opioid, rotation to a second strong opioid often restores analgesia with tolerable side effects (Smith, 2008). The clinical success of 'opioid rotation' suggests the possibility of subtle between-opioid differences in their *in vivo* efficacy and adverse-event profiles (Smith, 2008). Hence, the present study in rats was designed to assess qualitative and quantitative between-opioid differences in the *in vivo* antinociception, constipation and respiratory depressant profiles of seven commonly used opioid agonists, namely morphine, its analgesically active metabolite, morphine-6-glucuronide (M6G), oxycodone, fentanyl, buprenorphine, DPDPE ([D-penicillamine^{2,5}]-enkephalin) and U69,593.

To avoid the potentially confounding effects of drug metabolism associated with the systemic dosing route, the *in vivo* pharmacodynamic profiles of these seven opioids were assessed using bolus doses delivered i.c.v., as there is a very little drug metabolism when this route is used (Misra *et al.*, 2003). Additionally, injection into the lateral ventricle delivers the opioid ligands in close proximity to the periaqueductal grey matter, an area of the brain with a high density of opioid receptors (Porreca *et al.*, 1984; Pham *et al.*, 2003). As the CSF freely exchanges molecules with the extracellular fluid of the brain parenchyma, this enables therapeutic CNS concentrations to be attained after i.c.v. dosing (Misra *et al.*, 2003). Indeed, i.c.v. administration of clinically available opioid analgesics produces potent antinociception and analgesia in rodents and humans respectively (Blond *et al.*, 1994; Ross and Smith, 1997; Nielsen *et al.*, 2000).

Following i.c.v. administration in the rat, drugs flow in a net unidirectional manner in the CSF from the lateral ventricle to the third ventricle, to the fourth ventricle and into the subarachnoid space and/or central canal of the spinal cord, into the venous sinuses and from there to the internal jugular vein (Levinger, 1971; Bui *et al.*, 1999). Interestingly, using digital subtraction radiography with densitometry for 90 min after injection of contrast agent via a chronically implanted cannula into the lateral ventricle of the rat brain, the radiographic density of the contrast agent was found to be 100% in the ventricles and cerebral aqueduct relative to the cannula tip (arbitrarily defined as 100%), with caudal spread to the upper cervical region (32%) and faint spreading to the lower cervical spine (7.9%) (Luger *et al.*, 2005). These findings were verified by serial cryosectioning (Luger *et al.*, 2005). Hence, following i.c.v. opioid administration in rats as we have used here, the extent to which peripheral gastrointestinal (GI) tract effects would be expected to contribute to constipation is low, compared with those of central origin. In the CNS, opioid analgesics such as morphine alter autonomic outflow to the small intestine (Galligan and Burks, 1983), which, in turn, reduces GI transit by increasing sphincter tone and decreasing propulsive peristaltic contractions in both the small and the large intestines (Ferrante, 1996; Meert and Vermeirsch, 2005). This delay in passage of intestinal contents allows greater absorption of water in the large intestine, increasing both viscosity and desiccation of intestinal contents (Meert and Vermeirsch, 2005).

Hence, in the present study, groups of adult male Sprague Dawley (SD) rats received single i.c.v. bolus doses of one of the seven opioid ligands listed above. Antinociception, constipation and respiratory depression were assessed using the warm water tail-flick test, the castor oil-induced diarrhoea test and whole body plethysmography (WBP) in awake, freely moving rats respectively.

Our findings show for the first time that following single i.c.v. bolus dose administration of one of the seven opioid agonists examined in drug-naïve adult male rats, no two had the same profile for producing antinociception, constipation and respiratory depression. Our findings, together with other recent work from our laboratory (Varamini *et al.*, 2012), collectively suggest that these pharmacodynamic endpoints are differentially regulated and that discovery of novel strong opioid analgesics with reduced propensity to produce constipation and/or respiratory depression will be possible.

Additionally, the subtle between-opioid differences documented in rats is likely to explain the success of 'opioid rotation' in the clinical setting whereby changing the treatment of patients experiencing inadequate analgesia and intolerable opioid-related side effects with one strong opioid, to a second strong opioid, often results in successful restoration of pain relief with acceptable tolerability.

Methods

Experimental animals

All animal care and experimental procedures complied with the Australia Code of Practice for the Care and Use of Animals for Scientific Purposes (7th edition, 2004) and were approved by the Animal Ethics Committee of The University of Queensland. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). A total of 752 SD rats were utilized in this study, encompassing 109 treatment cohorts (Table 1).

Male SD rats (Herston Medical Research Centre, Herston, Qld, Australia) weighing 140–180 g (6–7 weeks of age) upon arrival were housed in a purpose-built Physical Containment Level 2 (PC2) animal holding facility in groups of two to three

Table 1

Summary of the number of cohorts of adult male SD rats used to define the comparative pharmacological profiles of the seven selected opioid ligands

	Antinociception	Constipation	Respiratory depression
Morphine	6 cohorts	8 cohorts	6 cohorts
M6G	6 cohorts	4 cohorts	4 cohorts
Oxycodone	4 cohorts	5 cohorts	6 cohorts
Fentanyl	4 cohorts	4 cohorts	6 cohorts
Buprenorphine	6 cohorts	6 cohorts	4 cohorts
DPDPE	4 cohorts	4 cohorts	5 cohorts
U69,593	8 cohorts	5 cohorts	4 cohorts
Total cohorts[a]	38 cohorts	36 cohorts	35 cohorts

[a] n = 4–8 rats with chronically implanted i.c.v. guide cannulae per cohort.

in individually ventilated cages (BioZone, Thorne Hill, Ramsgate, Kent, UK) with rat chow (Specialty Feeds, Glen Forrest, WA, Australia) and tap water available *ad libitum* throughout the housing period. Rats were maintained in cages that contained recycled paper bedding material (FibreCycle Pty Ltd, Yatala, Qld, Australia) and environmental enrichment in the form of Kimwipes (Kimberly-Clark Professional, Milsons Point, NSW, Australia) and Rat Chewsticks (Able Scientific, Welshpool, WA, Australia) until their weight reached 200–220 g, the weight range for surgical guide cannula implantation. After surgery, rats were housed individually. Housing conditions used a 12 h/12 h light/dark cycle and a mean (±SEM) room temperature set at 23 (±3)°C. Each rat was tested on only one occasion. Rats with a surgically implanted i.c.v. guide cannula were allocated randomly to receive active drug or vehicle in each dosing cohort.

The same investigator performed all of the anaesthesia, i.c.v. cannula implantation surgery, monitoring of animals post-surgery, drug dosing and behavioural testing. To minimize bias in the conduct of each behavioural test, objective quantitative endpoints rather than subjective endpoints were measured. Specifically, the following endpoints were measured: (i) tail-flick latency, which is the time (in seconds) for a rat to flick its tail in response to an applied noxious heat stimulus; (ii) the presence or absence of diarrhoea in rats at specific pre-specified time points after oral castor oil administration; and (iii) quantitative effects on respiratory parameters (tidal volume, frequency and minute ventilation) in awake, freely moving rats using computer-controlled WBP (Buxco Electronics, Troy, NY, USA) and the BioSystem XA software package (ver. 2.11.2; Buxco Electronics).

All behavioural tests were carried out in the light phase between 0800 h and 1830 h.

Implantation of i.c.v. guide cannula

After weighing, individual rats were anaesthetized using a mixture of xylazine (8 m·kg^{-1}; Troy Laboratories Pty Ltd, Smithfield, NSW, Australia) and zoletil (50 mg·kg^{-1}; Virbac Australia Pty Limited, Milperra, NSW, Australia). Animals were administered a s.c. injection of benzylpenicillin at 60 mg·kg^{-1} (CSL Corporate, Parkville, Vic, Australia) as antibiotic prophylaxis. Once each rat was deeply anaesthetized, the head was shaved and secured in position in a stereotaxic frame using the ear bars and upper incisor bar (Kopf Instruments, Tujunga, CA, USA). A small mid-sagittal incision was made and bregma located as the anatomical reference point. A 21-gauge (21G) stainless steel guide cannula extending to 1 mm above the right lateral ventricle of the brain was implanted at 0.8 mm posterior; 1.5 mm lateral; 4.15 mm ventral, relative to bregma. A 25G stainless steel cannula plug was kept in the guide cannula except during drug injections. Guide cannulae were fixed in position with acrylic dental cement (Metrodent Limited, Huddersfield, West Yorkshire, UK) and the incision closed using 5-0 Dysilk (Dynek Pty Ltd, Hendon, SA, Australia). Topical antibiotic powder (Tricin; Jurox Pty Ltd, Rutherford, NSW, Australia) was applied to the closed incision. Rats were kept warm and monitored closely, based upon their body weight and clinical signs during post-surgical recovery.

Intracerebroventricular drug administration

Following a 5–7 day recovery period after surgical implantation of the stainless steel guide cannula, individual rats received single i.c.v. bolus doses of one opioid or vehicle into the right lateral ventricle of the brain. Briefly, rats were lightly anaesthetized with a mixture of 50% O_2 and 50% CO_2 or 3% isoflurane (Abbott Australasia Pty Ltd, Botany, NSW, Australia) delivered in oxygen to facilitate i.c.v. drug administration. Injections were made using a 5 µL syringe (SGE Analytical Science Pty Ltd, Ringwood, Vic, Australia) at a rate of approximately 10 µL per 30 s.

Following completion of experimentation, correct guide cannula placement was assessed in individual rats by injection of 2.5 µL of malachite green dye solution (0.25 mg·mL^{-1}; BDH Chemicals Ltd, Poole, UK) while rats were anaesthetized using a 50%/50% mixture of O_2 and CO_2. Rats were exposed to 100% CO_2 and the decapitated, the brain removed and cut coronally at the point of entry of the guide cannula. Correct cannula placement was verified by appearance of dye in the lumen of the lateral ventricle, often with spread of dye into the third ventricle. Data for animals where dye did not meet these criteria were excluded from the analysis.

Assessment of antinociception: warm water tail-flick test

Opioid agonist-induced antinociception in rats was assessed using the warm water tail-flick test with a water temperature of 52.5°C. Rats were acclimatized in rodent restrainers (SDR Clinical Technology, Middle Cove, NSW, Australia) for at least 30 min prior to baseline tail-flick readings. Baseline tail-flick measurement comprised three consecutive baseline readings at 5 min intervals that did not deviate by more than 0.5 s from each other. The tail-flick response was elicited by immersion of the distal 2.5 cm of the rat tail into the warm water. A maximum cut-off time of 10 s was used to avoid tissue damage. Antinociception was quantified by measuring tail-flick latencies just prior to dosing and at the following post-dosing times: 7, 15, 30, 45, 60, 75, 90, 120 and 180 min.

For antinociception, 263 rats with a chronically implanted i.c.v. guide cannula were allocated randomly to dosing cohorts that collectively comprised 27 experimental and 2 control groups. For each of the 38 dosing cohorts (Table 1), at least one rat received vehicle.

Assessment of constipation: inhibition of castor oil-induced diarrhoea

Prior to experimentation, rats were placed individually in wire mesh observation chambers and fasted for 16 h with free access to water. At the commencement of experimentation, the observation chambers containing the rats were placed on the experimental stations beneath which were pre-weighed underlay sheets for each chamber. Animals were allowed a 1 h acclimatization period prior to i.c.v. administration of opioid or vehicle. At 5 min post-dosing, individual rats received 1 mL of castor oil (Sigma-Aldrich, Castle Hill, NSW, Australia) by gavage and then observed for castor oil-induced diarrhoea at the following post-dosing times, viz., 1, 2, 3, 4, 5, 6, 7 and 8 h. At each time of assessment, the following parameters were recorded: (i) weight of faeces (0 = no faeces, 1 = 0.1–1 4 g, 2 = 1.5–3 g, 3 ≥ 3 g of faeces); and (ii) faeces consistency (0 = normal faeces, 1 = well-shaped wet faeces, 2 = shapeless faeces, 3 = shapeless faeces with large amounts of liquid). Diarrhoea was defined as present when both weight and consistency of faeces were scored at ≥2. As opioid agonists inhibit castor oil-induced diarrhoea in rats, the time during which there was inhibition of castor oil-induced diarrhoea was measured as an index of constipation.

For constipation, 256 rats with a chronically implanted i.c.v. guide cannula were allocated randomly to dosing cohorts that collectively comprised 26 experimental and 2 control groups. For each of the 36 dosing cohorts (Table 1), at least one rat received vehicle.

Respiratory depression: WBP

At 5–7 days after i.c.v. guide cannula implantation, conscious SD rats were placed individually within WBP chambers. The frequency of respiration, tidal volume and minute ventilation were recorded after rats were exposed to normal atmospheric air (baseline) or to 5 min epochs of a hypercapnic gas mixture (8% CO_2, 21% O_2 and 71% N_2) prior to and after i.c.v. opioid or vehicle administration. WBP in conscious, unrestrained rodents is widely utilized to assess ventilatory function (Goineau *et al.*, 2010) and for the evaluation of potential ventilatory depressant side effects of new drugs in development (Goineau *et al.*, 2010). Inclusion of 8% CO_2 in the inspired air (hypercapnic challenge) produces a reliable stimulation of ventilation in control rats (van den Hoogen and Colpaert, 1986).

The mean change in minute ventilation (ΔMV) for rats exposed to normal atmospheric air relative to the same animals exposed to the hypercapnic gas mixture, prior to administration of i.c.v. bolus doses of opioid or vehicle, was set as a baseline value of 100%. Following i.c.v. opioid or vehicle administration, minute ventilation (MV) recorded continuously for 1 min while rats breathed atmospheric air followed by a 5 min hypercapnia challenge with the final minute used to calculate ΔMV. Rats then had a 9 min recovery period breathing atmospheric air. This procedure was repeated every 15 min for 2 h post-i.c.v. vehicle or opioid administration (Figure 1).

For respiratory depression, 233 rats with a chronically implanted i.c.v. guide cannula were allocated randomly to dosing cohorts that collectively comprised 25 experimental and 2 control groups. For each of the 35 dosing cohorts (Table 1), at least one rat received vehicle.

Data analysis: antinociception

For antinociception in individual rats, tail-flick latencies were normalized to the percentage of the maximum possible effect (%MPE), using the following formula:

$$\%\text{MPE} = \frac{\text{Post-drug latency} - \text{Pre-drug latency}}{\text{Maxlatency} - \text{Pre-drug latency}} \times 100$$

All data are presented as mean (±SEM) for the particular treatment group, unless otherwise specified. Dose–response curves were produced by plotting mean peak (±SEM) %MPE values versus log dose, and the mean dose that produced half-maximal antinociception (ED_{50}) was estimated using non-linear regression as implemented in the GraphPad™ Prism software program (ver. 5.03; GraphPad Software, San Diego, CA, USA). For antinociception, full and partial agonists are defined as treatments that evoke a mean peak effect of ≥75%MPE, or <75%MPE with a ceiling effect evident respectively.

Data analysis: constipation

For constipation in individual rats, the percentage of animals with diarrhoea (%AD) versus time curve was plotted for each opioid dose or vehicle. The ED_{50} values were estimated based upon the percentage of animals with diarrhoea at 3 h post-dosing using non-linear regression as implemented in the GraphPad Prism software program (ver. 5.03; GraphPad Software) versus log dose values. For constipation, full and partial agonists are defined as treatments that produce values for percentage of animals with diarrhoea of ≤50% at 4 h, or >50% at 4 h with a ceiling effect evident respectively. An inactive treatment was defined as producing 100% animals with diarrhoea at 2 h, in a manner similar to vehicle.

Data analysis: respiratory depression

For respiratory depression in individual rats, the results were plotted as mean (±SEM) '% depression of minute ventilation relative to pre-treatment (%DEP)' versus time curves. The

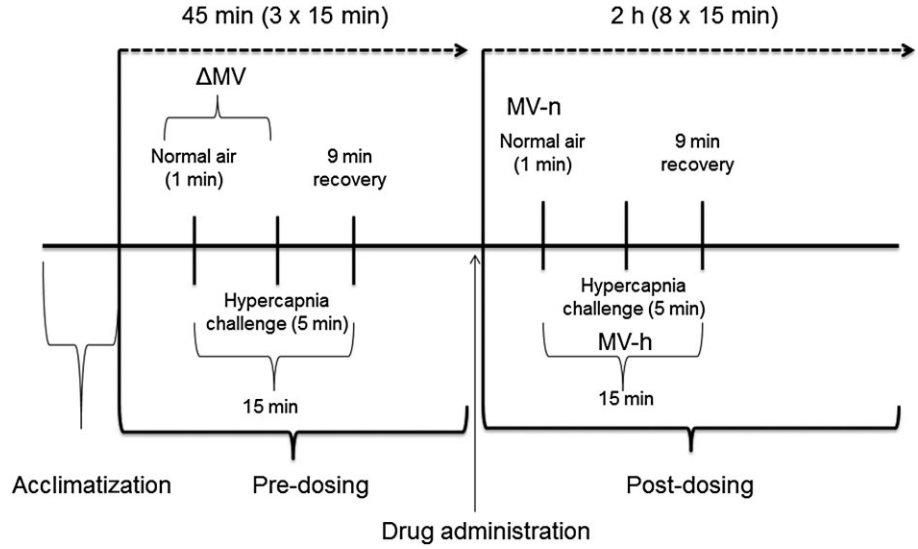

Figure 1

Schematic representation of the WBP method used to assess respiratory function in conscious, unrestrained rats. Rats were placed individually in WBP chambers and acclimatized for ~30–45 min. This was followed by pre-dosing measurement of minute ventilation (MV) while animals were breathing normal atmospheric air (MV-n). This, in turn, was followed by a 5 min exposure period to a hypercapnic gas mixture (MV-h) followed by breathing normal air. Recordings of pre-dosing MV-n and MV-h were repeated twice and the difference in minute ventilation (ΔMV) between pre-dosing MV-n and MV-h was calculated. The pre-dosing mean ΔMV value served as the baseline for comparison with the post-dosing ΔMV. Eight cycles of post-dosing MV-n and MV-h values were recorded at 15 min intervals for a total period of 2 h.

extent and duration of respiratory depression was calculated as the 'Area under the %DEP versus time curve (%DEP-AUC)' over a 2 h post-dosing period (%DEP.h) for individual rats. The ED_{50} values were estimated using non-linear regression as implemented in the GraphPad Prism software program (ver. 5.03; GraphPad Software) from the peak %DEP versus log dose data. For respiratory depression, full and partial agonists are defined as treatments that evoke a mean peak effect of ≥70%DEP, or <70%DEP with a ceiling effect evident respectively.

Statistical analysis

Statistical comparisons between opioid and vehicle-treated groups were performed using ANOVA followed by Dunnett's *post hoc* test. The statistical significance criterion was $P < 0.05$.

Materials

Morphine hydrochloride and oxycodone hydrochloride were purchased from the Royal Brisbane and Women's Hospital pharmacy (Herston, Qld, Australia). Morphine-6-β-D-glucuronide hydrate (M6G), fentanyl (*N*-phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]-propanamide citrate salt), buprenorphine ([5α,7α(*S*)]-17-(cyclopropylmethyl)-α-(1,1-dimethylethyl)4,5-epoxy-18,19-dihydo-3-hydroxy-6-methoxy-α-methyl-6,14-ethenomorphinan-7-methanol hydrochloride), DPDPE, ([D-penicillamine^{2,5}]-enkephalin) and U69,593 ((+)-[5α,7α,8β]-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide) were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). All opioids except U69,593 were dissolved in water for injection BP (Pfizer, West Ryde, NSW, Australia). The vehicle for U69,593 was 20% propylene glycol (Fronine Laboratory Supplies, Brisbane, Qld, Australia): 80% water for injection BP. Where applicable, drug doses were calculated based upon the hydrochloride or citrate salt weights. The seven opioid agonists were selected on the basis of their clinical relevance (morphine and its analgesically active metabolite, M6G, fentanyl, oxycodone and buprenorphine) for comparison with DPDPE and U69,593 that are regarded as having DOP and KOP receptor selectivity, respectively, on the basis of their *in vitro* opioid receptor binding and functional activity profiles (Katsumata *et al.*, 1995; Toll *et al.*, 1998).

Results

Morphine

Antinociception. After i.c.v. bolus dose administration of morphine, dose-dependent antinociception was evoked, characterized by two phases with the first peak at 30–60 min post-dosing and the second peak at 75–120 min post-dosing (Figure 2), whereas vehicle was inactive (Figure 3). The mean duration of action for i.c.v. morphine was in the range of 2 to >3 h. The mean (with 95% CI) ED_{50} for i.c.v. morphine, based upon peak %MPE values, is shown in Table 2.

Constipation. Administration of i.c.v. morphine at 30–150 nmol evoked dose-dependent inhibition of castor oil-induced diarrhoea (Figure 4). However, at i.c.v. doses of 300 and 450 nmol, the anti-diarrhoeal effects were less than that evoked by i.c.v. morphine at 150 nmol such that the dose–response relationship was bell shaped (Figure 5). Indeed, the effect of i.c.v. morphine at 450 nmol was not significantly (*P*

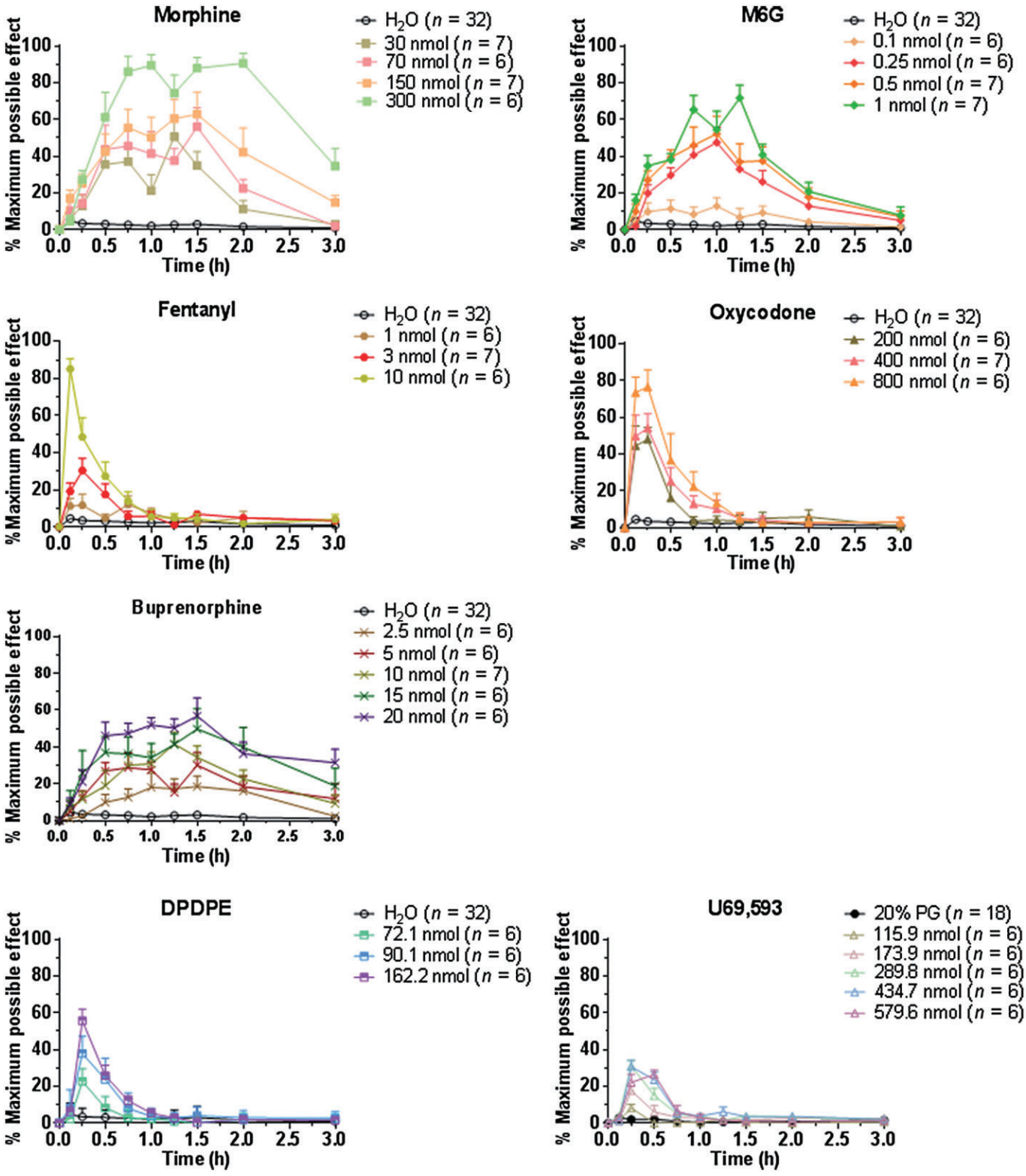

Figure 2

Dose-dependent antinociception evoked by i.c.v. bolus doses of each of the seven opioid agonists tested, assessed using the warm water 52.5°C tail-flick test in SD rats. Tail-flick latencies are plotted as the mean (±SEM) percentage maximum possible effect (%MPE) over a 3 h post-dosing period. Drugs were dissolved in either water for injection (H_2O) or 20% propylene glycol in H_2O (20% PG).

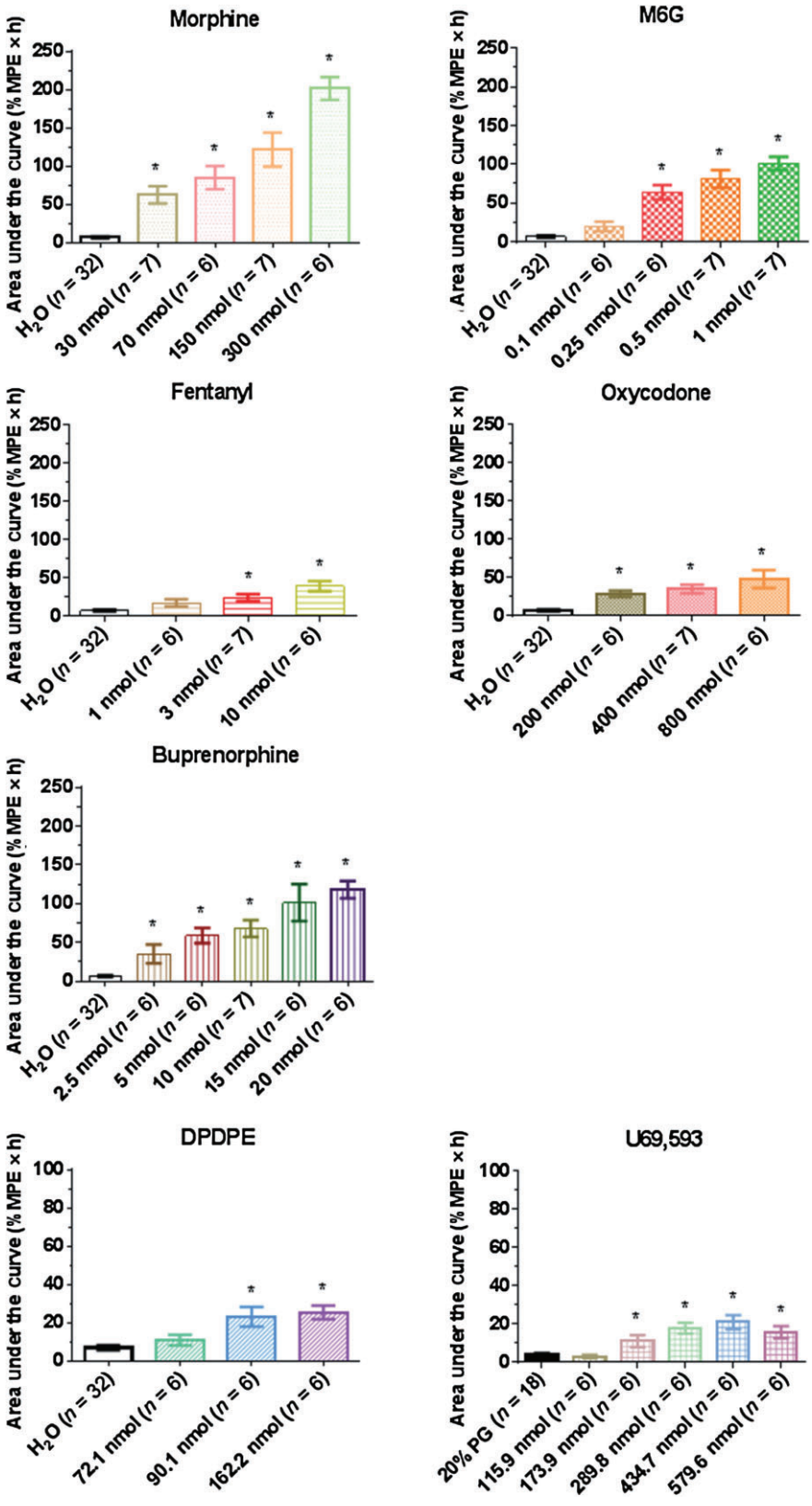

Figure 3

Mean (±SEM) extent and duration of antinociception (%MPE-AUC) evoked by i.c.v. bolus doses of individual opioids assessed using the warm water (52.5°C) tail-flick test in SD rats over a 3 h post-dosing period. Control rats were administered i.c.v. bolus doses of vehicle that was either H_2O or 20% propylene glycol (20% PG). *$P < 0.05$, significantly different from the vehicle control.

Table 2

Mean (with 95% CI) ED_{50} values for i.c.v. bolus doses of seven opioid agonists tested for evoking antinociception, constipation and respiratory depression in male SD rats

	Mean (95% CI) ED_{50} values (nmol)		
Opioid	**Antinociception**	**Constipation**	**Respiratory depression**
Morphine	52.2 (27.6–98.5)	111.5 (111.4–111.7)[a]	88.5 (39.7–197.4)
M6G	0.4 (0.28–0.69)	0.61 (0.6–0.62)	0.3 (0.2–0.5)
Oxycodone	287.9 (199.2–416.2)	355.6 (335.4–377.1)[b]	ND
Fentanyl	4.9 (1.53–15.7)	9.9 (9.6–10.2)	13.9 (10.0–19.3)
Buprenorphine	~20 (13.0–31.7)[b]	~7.5[b]	12.3 (8.7–17.4)
DPDPE	160.1 (96.5–265.6)	ND	ND
U69,593	ND[b]	ND	ND

[a]Due to the bell-shaped dose–response curve for morphine in the constipation assay, the ED_{50} was estimated using doses up to 150 nmol; higher doses were not included. [b]A ceiling effect was observed; the ED_{50} was estimated using doses up to that which produced the maximal effect; data from higher doses were not included. ND, not determinable.

> 0.05) different from that evoked by i.c.v. vehicle (Figure 5). The mean (with 95% CI) ED_{50} for i.c.v. morphine in the dose range, 30–150 nmol, based upon percentage of animals with diarrhoea at 3 h post-dosing, is given in Table 2.

Respiratory depression. Intracerebroventricular morphine at 70–450 nmol evoked dose-dependent respiratory depression (Figures 6 and 7), with the mean peak effect (%DEP) observed at 0.75–1.0 h post-dosing (Figure 6). At the highest dose tested (450 nmol), the mean duration of action was prolonged at >2 h. The estimated mean (95% CI) ED_{50} based upon peak %DEP values is shown in Table 2.

M6G

Antinociception

Administration of i.c.v. M6G at 0.1–1 nmol to rats evoked dose-dependent antinociception with peak effects at ~1 h post-dosing (Figure 2); mean ED_{50} based upon peak effect is shown in Table 2. At the highest tolerable dose (1 nmol), the mean duration of action was ~3 h. Increasing the dose of M6G to 2 nmol had poor tolerability due to neuroexcitatory behaviour requiring killing of the animals.

Constipation

Intracerebroventricular M6G produced dose-dependent inhibition of castor oil-induced diarrhoea and its potency was ~200-fold higher than that of morphine (Figure 4); mean ED_{50} based upon percentage of animals with diarrhoea at 3 h post-dosing is shown in Table 2. At the highest tolerable dose (1 nmol), M6G evoked a long-lasting anti-diarrhoeal effect such that it was not until 8 h that all animals had developed diarrhoea (Figure 4).

Respiratory depression

Administration of i.c.v. M6G at 0.1–1 nmol produced dose-dependent respiratory depression and its potency was ~250-fold higher than that of morphine (Figure 4). Mean peak effects (%DEP) were seen at 0.5–1 h post-dosing (Figure 6), and at 1 nmol, the duration of action was prolonged (Figure 6); mean (95% CI) ED_{50} is shown in Table 2.

Fentanyl

Antinociception. Administration of i.c.v. fentanyl at 1–10 nmol in rats produced dose-dependent antinociception with the mean peak effect at ~7 min post-dosing (Figure 2); for mean ED_{50} based upon peak antinociception, see Table 2. The mean durations of action were 0.75–1.0 h, showing that i.c.v. fentanyl is a 'fast on, fast off' opioid agonist, in contrast to morphine and M6G that evoked 'slow on, slow off' antinociception.

Constipation. Intracerebroventricular fentanyl at 1–10 nmol evoked dose-dependent inhibition of castor oil-induced diarrhoea (Figures 4 and 5). At the highest dose tested (10 nmol), fentanyl produced long-lasting constipation such that only 50% of animals had developed diarrhoea by 4 h post-dosing with 100% by 6 h (Figure 4); for mean ED_{50} based upon percentage of animals with diarrhoea at 3 h post-dosing, see Table 2.

Respiratory depression. Following i.c.v. fentanyl at 3–30 nmol, there was dose-dependent respiratory depression with the mean peak effect at ~15 min post-dosing (Figure 6); for mean ED_{50} values, see Table 2. The mean durations of action were ~1–1.5 h, showing that i.c.v. fentanyl is a 'fast on, fast off' opioid for respiratory depression in contrast to morphine and M6G that exhibited 'slow on, slow off' respiratory depression.

Oxycodone

Antinociception. Similar to fentanyl, i.c.v. oxycodone at 200–800 nmol evoked dose-dependent antinociception in rats characterized by a single antinociceptive phase with peak antinociception achieved by ~7 min post-dosing (Figures 2

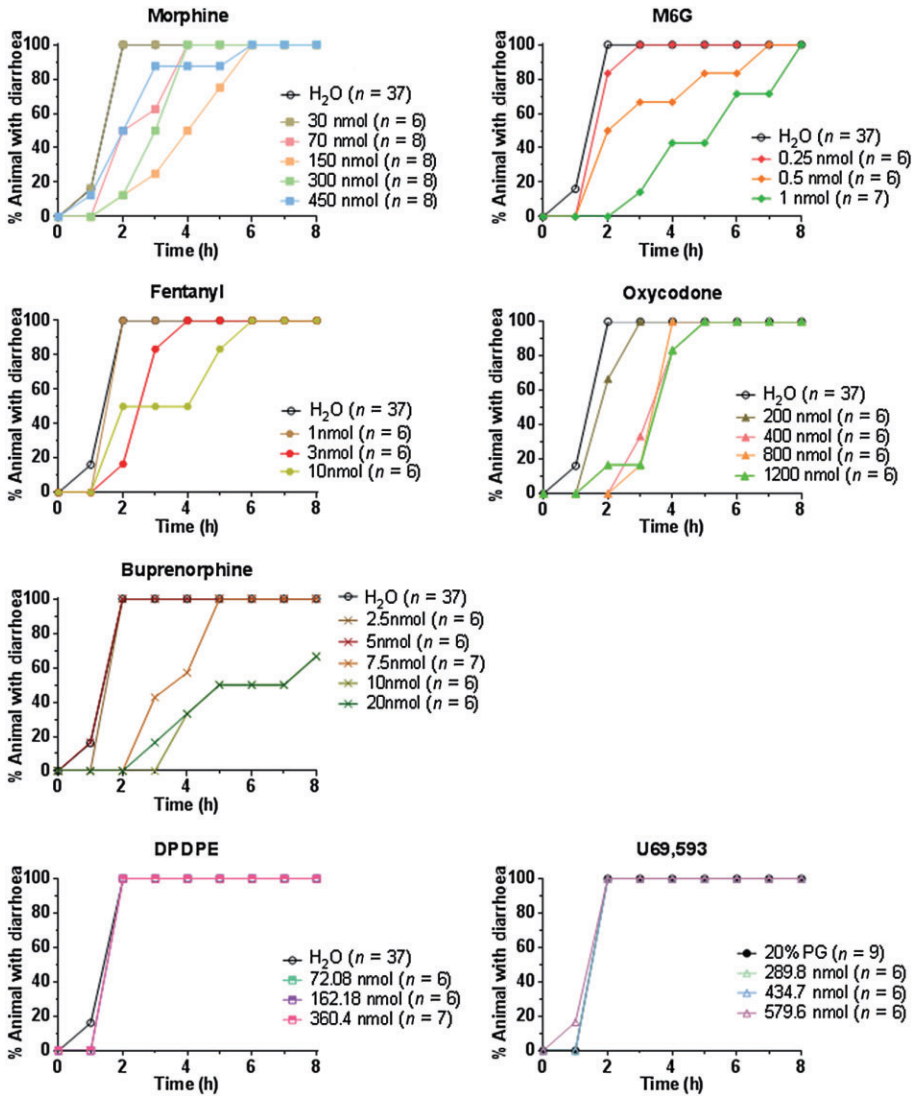

Figure 4

Effects of i.c.v. bolus doses of each of the seven opioid agonists tested on castor oil-induced diarrhoea in rats. Data are presented as the percentage of animals with castor oil-induced diarrhoea versus time over an 8 h post-dosing interval. Control rats were administered i.c.v. bolus doses of vehicle, viz., H_2O or 20% propylene glycol (20% PG).

and 3). The mean duration of action was ~0.75–1.25 h and the mean ED_{50} (95% CI) is given in Table 2.

Constipation. Intracerebroventricular oxycodone at 400 but not 200 nmol produced a significant ($P < 0.05$) anti-diarrhoeal effect (Figure 5); for the mean ED_{50} based upon percentage of animals with diarrhoea at 3 h post-dosing, see Table 2. However, increasing the i.c.v. dose to 800 and 1200 nmol did not further increase the anti-diarrhoeal effect (Figure 5), showing that i.c.v. oxycodone is a partial agonist for constipation.

Respiratory depression. Similar to fentanyl, i.c.v. oxycodone produced dose-dependent respiratory depression characterized by a rapid onset of action with mean peak effects at 15 min post-dosing (Figure 6). Unique among the seven opioids assessed herein, i.c.v. oxycodone was a partial agonist for respiratory depression with a ceiling effect evident for doses in the range 200–2000 nmol (Figure 7). Due to the pronounced ceiling effect, an ED_{50} could not be determined (Table 2).

Buprenorphine

Antinociception. Intracerebroventricular buprenorphine at 2.5–15 nmol produced dose-dependent antinociception in rats with the mean peak effect at 0.75–1.5 h post-dosing (Figures 2 and 3); a ceiling effect was evident at 20 nmol (Figure 2); mean ED_{50} is shown in Table 2. The mean duration of action was ≥3 h, showing that buprenorphine was a 'slow on, slow off' opioid for antinociception (Figure 2).

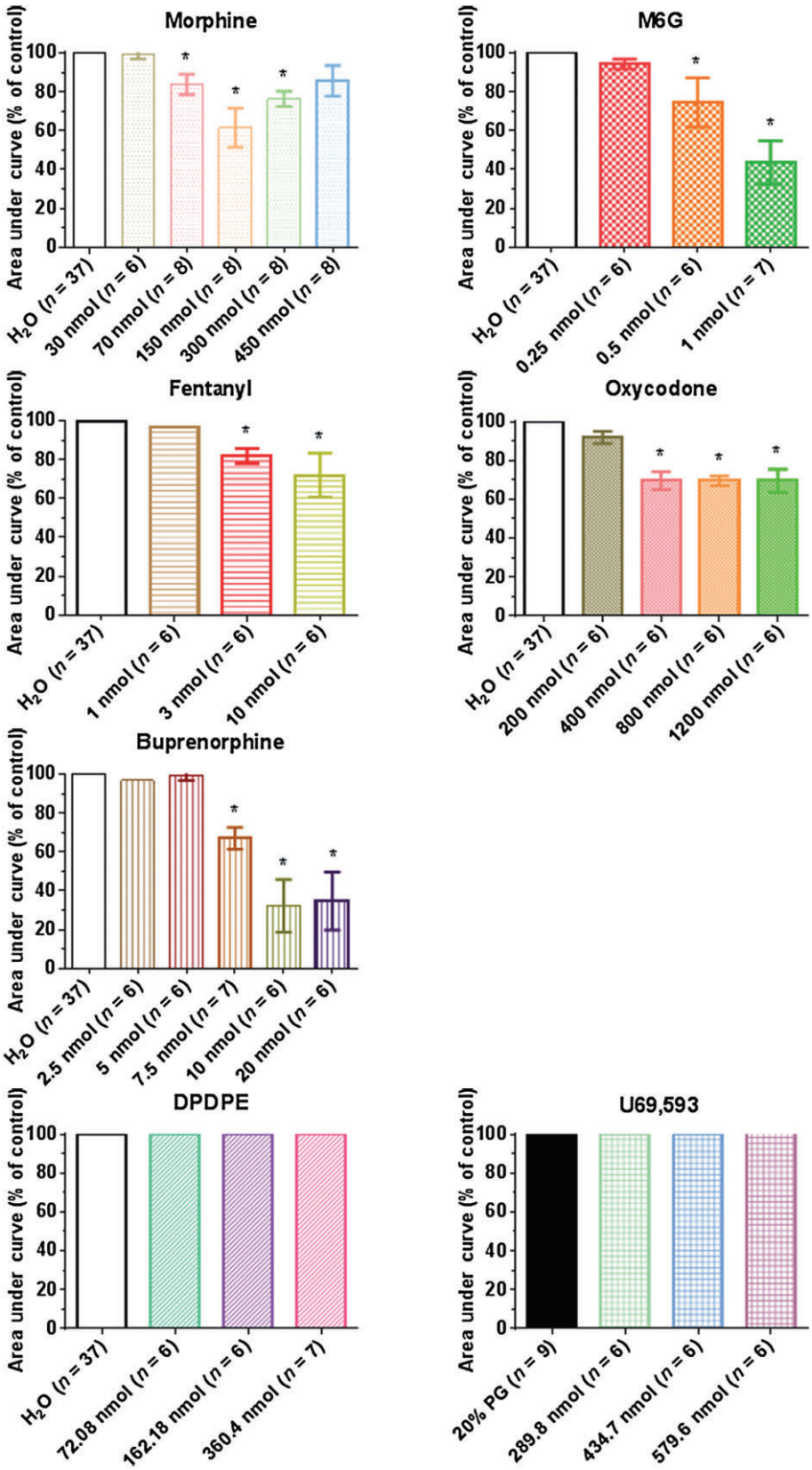

Figure 5

Effect of single i.c.v. bolus doses of the opioid agonists tested on castor oil-induced diarrhoea in male SD rats. Data are shown as mean (±SEM) area under the curve (AUC) for % animals with diarrhoea (% of control) versus time over an 8 h post-dosing interval. Vehicle comprised water for injection (H_2O) or 20% propylene glycol (20% PG). *$P < 0.05$, significantly different from the vehicle control.

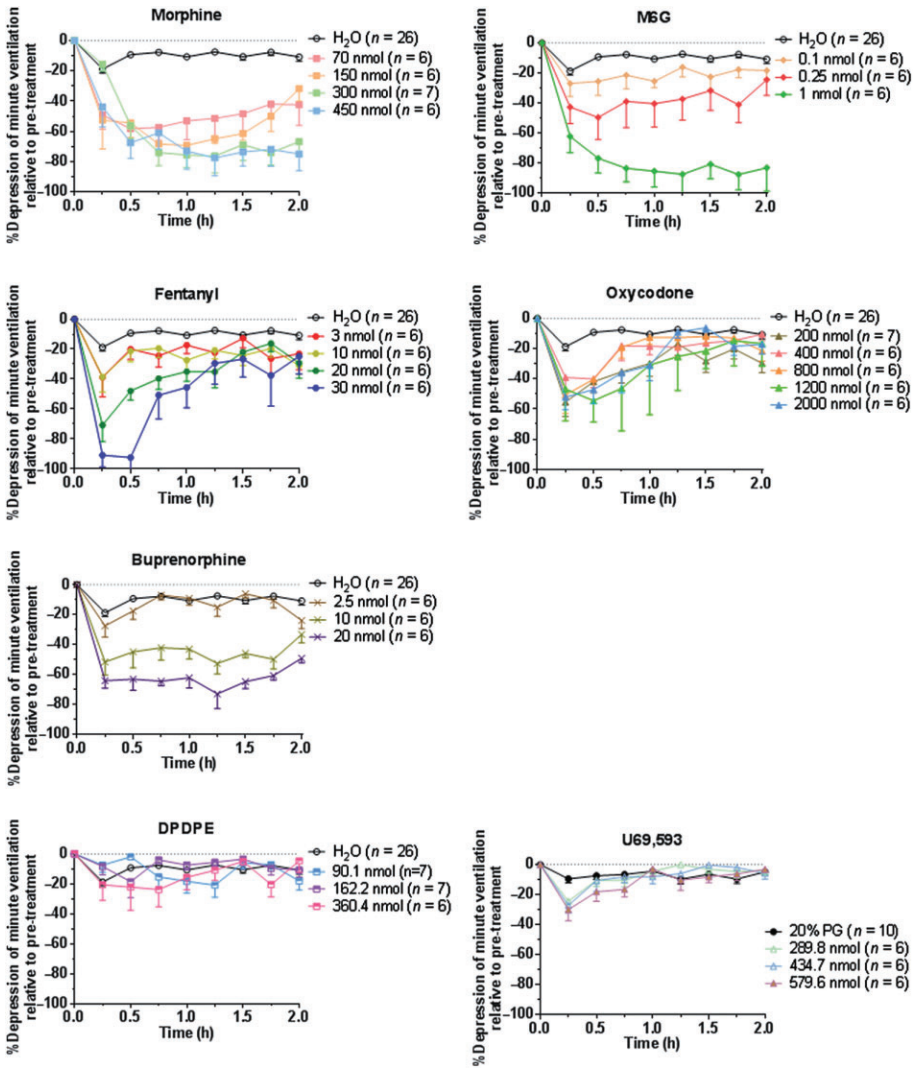

Figure 6

Respiratory depressant effects evoked by i.c.v. bolus doses of each of the seven opioid agonists tested, assessed using WBP and hypercapnic challenge in conscious male SD rats. Data are presented as mean (±SEM) percentage (%) depression of minute ventilation relative to that determined in the pre-treatment period, for eight cycles over a 2 h post-dosing period. Control rats were administered i.c.v. bolus doses of vehicle, viz., H_2O or 20% propylene glycol (20% PG).

Constipation. Bolus doses of i.c.v. buprenorphine produced dose-dependent inhibition of castor oil-induced diarrhoea (Figure 4). The anti-diarrhoeal effect at 7.5 nmol was comparable with that of i.c.v. morphine at 150 nmol (Figure 4); mean ED_{50} is shown in Table 2. At i.c.v. doses of 10 and 20 nmol, long-lasting constipation was produced as only ~70% of rats had developed diarrhoea by completion of the 8 h experimental period (Figure 4).

Respiratory depression. Intracerebroventricular buprenorphine at 2.5–20 nmol evoked dose-dependent respiratory depression with the mean peak effect at 15 min post-dosing (Figure 6); mean ED_{50} is shown in Table 2. For doses at 10 and 20 nmol, the mean duration of action was >2 h.

DPDPE

Antinociception

Bolus doses of i.c.v. DPDPE at 72.1–162.2 nmol produced dose-dependent antinociception in rats with the mean peak effect at 15 min post-dosing (Figure 2), similar to fentanyl and oxycodone. The corresponding mean duration of action was <1 h and the mean ED_{50} is shown in Table 2.

Constipation

Intracerebroventricular DPDPE at 72.1–360.4 nmol produce insignificant anti-diarrhoeal effects ($P > 0.05$) in a manner similar to vehicle (Figure 5). Higher doses were not tested due to solubility issues, and so an ED_{50} was not able to be determined (Table 2).

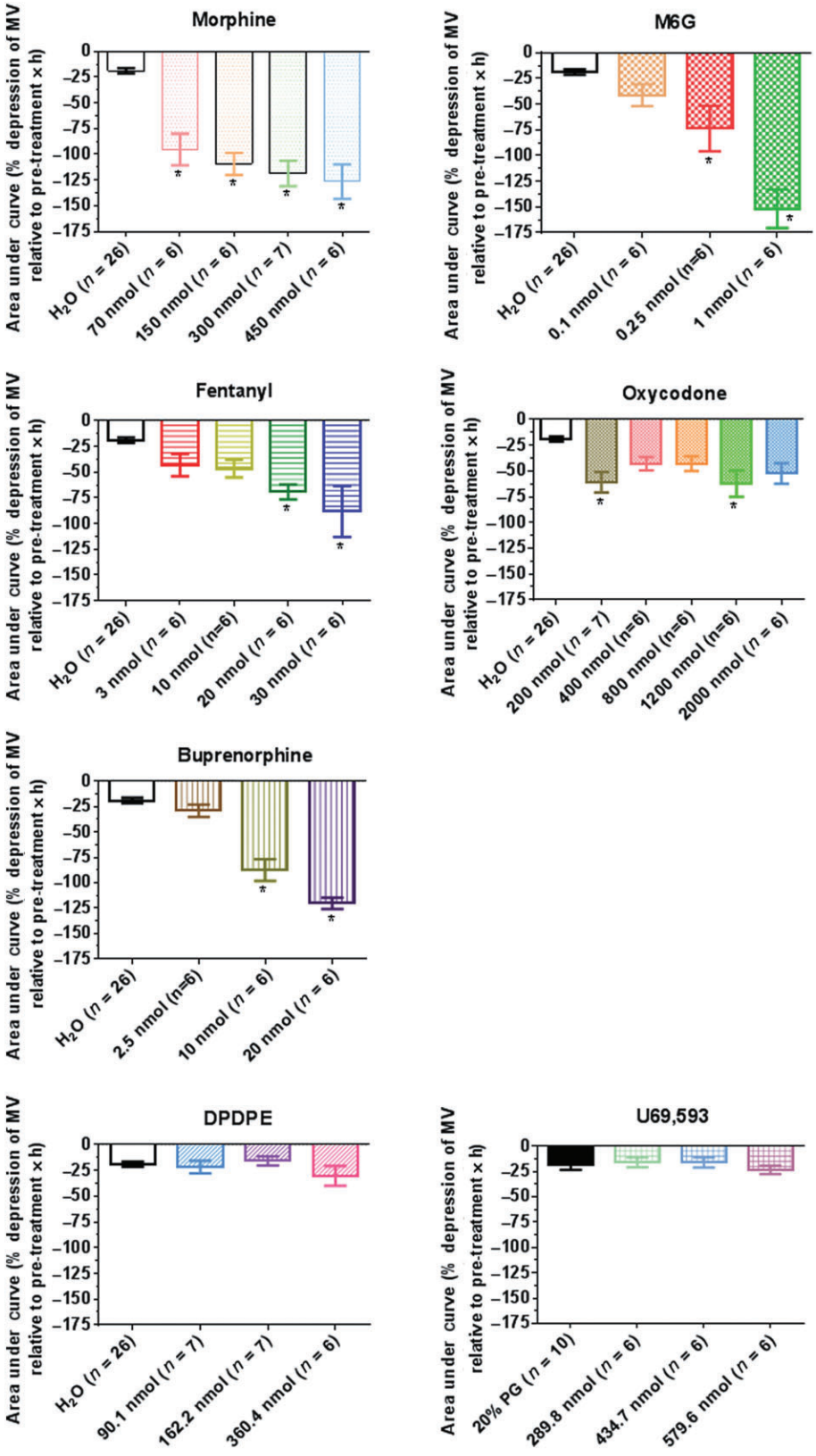

Figure 7

Mean (±SEM) extent and duration of respiratory depression (area under the curve for % depression of minute ventilation relative to pre-treatment versus time) evoked by i.c.v. bolus doses of individual opioids assessed usingWBP in male SD rats over a 2 h post-dosing period. Control rats received i.c.v. bolus doses of vehicle that was either H_2O or 20% propylene glycol (20% PG). *$P < 0.05$, significantly different from the vehicle control.

Respiratory depression

Administration of i.c.v. DPDPE at 90.0–360.4 nmol, insignificant respiratory depression ($P > 0.05$) was produced in a manner similar to vehicle (Figure 7). Hence, it was not possible to estimate an ED_{50} (Table 2).

U69,593

Antinociception

Intracerebroventricular administration of U69,593 evoked dose-dependent antinociception in rats characterized by a single peak effect at 7–30 min post-dosing, similar to other 'fast on, fast off' opioids (Figure 2). The mean durations of action were <1 h. U69,593 was a partial agonist for antinociception as there was a marked ceiling effect evident for doses ≥ 289.8 nmol (Figures 2 and 3). Hence, we could not determine the ED_{50}.

Constipation

At the doses tested, i.c.v. U69,593 produced insignificant inhibition of castor oil-induced diarrhoea (Figures 4 and 5). Larger doses were not administered due to solubility issues and so we could not determine the ED_{50}.

Respiratory depression

Similarly, at the doses tested, i.c.v. U69,593 produced insignificant respiratory depression (Figures 6 and 7) with solubility issues precluding the administration of larger doses. Hence, it was not possible to estimate the ED_{50}.

Discussion

Our findings show for the first time that following i.c.v. bolus dose administration of seven common opioid agonists in rats, no two have the same profile for evoking antinociception, constipation and respiratory depression (Table 3). Specifically, single i.c.v. bolus doses of morphine, fentanyl and oxycodone produced dose-dependent antinociception and were full agonists, as reported previously (Abbott and Palmour, 1988; Leow and Smith, 1994; Furst *et al.*, 2005). Although buprenorphine and M6G also produced dose-dependent antinociception, they were partial agonists on the basis of sub-maximal efficacy and prominent neuroexcitatory side effects respectively. The prototypic DOP and KOP receptor agonists, DPDPE and U69,593, produced dose-dependent antinociception, but U69,593 was a partial agonist. Together, our data show that opioid antinociception is mediated primarily by the MOP receptor, consistent with conclusions drawn from studies using MOP receptor knockout mice (Dietis *et al.*, 2011).

The antinociceptive potency of i.c.v. M6G in our experiments was ~10-fold higher than for fentanyl, 40-fold higher than for buprenorphine, 130-fold higher than for morphine and ~650-fold higher than for oxycodone. In conscious rats, there were marked between-opioid differences in the dose–response relationships for constipation and respiratory depression, relative to the corresponding antinociception profiles. For the seven opioids assessed, constipation and respiratory depression were mediated primarily via the MOP receptor as neither DPDPE nor U69,593 evoked constipation or respiratory depression.

Table 3

Potency rank order for the seven opioid ligands of interest for evoking antinociception, respiratory depression and constipation after i.c.v. bolus dose administration in rats

Opioid	Potency rank order
Morphine	Antinociception > respiratory depression > constipation[a]
M6G	Respiratory depression > antinociception > constipation
Oxycodone	Antinociception > constipation[b] > respiratory depression[b]
Fentanyl	Antinociception > constipation > respiratory depression
Buprenorphine	Constipation > respiratory depression > antinociception[c]
DPDPE	ND
U69,593	ND

[a]Dose-dependent anti-diarrhoeal effect as an index of constipation for doses up to 150 nmol that was progressively reversed by doses at 300 and 450 nmol. [b]Partial agonist for constipation and respiratory depression. [c]Partial agonist for antinociception. ND, not determinable.

For i.c.v. morphine, the potency rank order was antinociception > respiratory depression > constipation (Table 2), consistent with the previously reported higher potency of morphine for producing antinociception compared with respiratory depression (Ling *et al.*, 1985). Mean peak respiratory depression occurred at ~0.5–1 h post-dosing and the mean duration of action was >2 h, in agreement with others (Pazos and Florez, 1983). The widely held notion that morphine-evoked antinociception and respiratory depression are mechanistically inseparable (Romberg *et al.*, 2003) is discounted by observations in mice lacking β-arrestin-2, that respiratory depression was reduced whereas antinociception was enhanced (Raehal *et al.*, 2005).

Although i.c.v. morphine exhibited full agonism for antinociception and respiratory depression, its dose–response curve for constipation was bell shaped (Figure 5). Doses up to 150 nmol produced dose-dependent inhibition of castor oil-induced diarrhoea, whereas doses at 300 and 450 nmol produced progressive reversal of the anti-diarrhoeal activity. Our findings mirror the bell-shaped dose–response curve for i.c.v. morphine for inhibition of duodenal contraction frequency in rats (Galligan and Burks, 1983). The apparent reversal of the anti-diarrhoeal effect of i.c.v. morphine at the higher doses assessed (300 and 450 nmol) may be underpinned by significant caudal redistribution to activate spinal nociceptin opioid (NOP) receptors as their activation by nociceptin counteracts the effects of opioid agonists (Chung *et al.*, 2006) including the inhibitory effects of morphine on colonic contraction and transit (Taniguchi *et al.*, 1998). Conversely,

supraspinal NOP receptor activation by i.c.v. nociceptin evoked dose-dependent inhibition of colonic propulsive activity in mice (Osinski *et al.*, 1999). Alternatively, progressive reversal of the anti-diarrhoeal effects of morphine at the higher i.c.v. doses may be due to its partial agonism for β-arrestin-2 recruitment at the MOP receptor (Molinari *et al.*, 2010) and/or its antagonism of β-arrestin-2 recruitment at the DOP receptor (Molinari *et al.*, 2010). This possibility is supported by the marked reduction in morphine-induced constipation observed in β-arrestin-2 knockout compared with wild-type mice (Raehal *et al.*, 2005).

M6G was a partial agonist for antinociception in rats herein, but it was a full agonist for respiratory depression and constipation with these latter effects characterised by a prolonged duration of action at the higher doses tested. M6G's potency rank order was respiratory depression > antinociception > constipation (Table 3). For constipation, i.c.v. M6G was an order of magnitude more potent than buprenorphine and fentanyl, ~180-fold more potent than morphine and 580-fold higher more potent than oxycodone, whereas DPDPE and U69,593 were inactive (Table 2). For respiratory depression, M6G was ~4-fold more potent than buprenorphine and fentanyl, ~270-fold more potent than morphine and 600-fold more potent than oxycodone. Deaths due to opioid overdose are almost always due to respiratory depression (Francisco, 2007), making respiratory depression the most serious adverse effect associated with the use of opioid analgesics (Reisine and Pasternak, 1996; Otis, 1999). In patients given morphine by systemic routes, metabolically derived M6G crosses the blood–brain barrier (Carrupt *et al.*, 1991; Bourasset *et al.*, 2003), resulting in mean steady-state CSF M6G concentrations that are ~12% of the corresponding plasma concentrations (Smith *et al.*, 1999). As metabolically derived M6G has the potential to induce respiratory depression, caution is required in patients with renal impairment as M6G will accumulate in the systemic circulation and the CSF, increasing the likelihood of M6G-induced respiratory depression at usual morphine doses (Wright *et al.*, 1994; South *et al.*, 2001).

For i.c.v. buprenorphine, our experiments showed it was a partial agonist for antinociception, but a full agonist for constipation and respiratory depression, the potency rank order was constipation > respiratory depression > antinociception (Table 3). These findings differ from the general view that systemically administered buprenorphine is a partial agonist with fewer adverse effects than other opioids (Toll *et al.*, 1998; Lee *et al.*, 1999; Dahan, 2006; Brown *et al.*, 2011). A plausible explanation is that after systemic dosing, buprenorphine undergoes significant first-pass metabolism to norbuprenorphine that is analgesically inactive (Ohtani *et al.*, 1995), but it produces respiratory depression via a MOP receptor mechanism in the lungs that is opposed by buprenorphine itself (Ohtani *et al.*, 1997; Brown *et al.*, 2011). As brain parenchyma has a low capacity for drug metabolism, the respiratory depressant effects we found for i.c.v. buprenorphine were most likely due to buprenorphine alone.

Of the seven opioids assessed herein, i.c.v. fentanyl was the only full agonist for antinociception, constipation and respiratory depression. Fentanyl's 'fast on, fast off' antinociception and respiratory depression are attributed to its high lipophilicity (Mather and Smith, 1999). Although we found i.c.v. oxycodone was a full agonist for antinociception in rats, mirroring previous reports by our laboratory (Leow and Smith, 1994; Ross and Smith, 1997; Nielsen *et al.*, 2000), it was a partial agonist for constipation and respiratory depression. Despite oxycodone having relatively low lipophilicity with an octanol–water partition coefficient of 0.7 compared with 0.5 and 399 for morphine and fentanyl, respectively (Poyhia and Seppala, 1994), mean peak i.c.v. oxycodone respiratory depression was observed at 15 min post-dosing. The corresponding mean duration of action was short at ~0.75–1.25 h, mirroring i.c.v. fentanyl but contrasting with morphine despite oxycodone's lipophilicity being similar to morphine rather than fentanyl. Clinically, oxycodone produces potent analgesia after systemic but not epidural administration (Backlund *et al.*, 1997) with this latter characteristic being similar to the very low potency of intrathecal bolus doses of oxycodone (5–7% that of morphine) in rats (Plummer *et al.*, 1990; Lemberg *et al.*, 2006). Radioligand binding and functional assays in preparations from rodent tissues and cell-based opioid receptor expression systems show that oxycodone has relatively low MOP receptor affinity with insignificant DOP or KOP receptor affinity (Lalovic *et al.*, 2006; Nielsen *et al.*, 2007). However, previous work from our laboratory showed that oxycodone displaced [^{3}H]-DAMGO from both high and low affinity binding sites in rat brain membranes (Nielsen *et al.*, 2007) and that it bound with relatively high affinity at the pharmacologically defined κ_{2b}-opioid receptor subtype (Nielsen *et al.*, 2007). Interestingly, the DOP-KOP receptor heterodimer has an *in vitro* profile similar to the pharmacologically defined κ_2-opioid receptor (Jordan and Devi, 1999). Hence, oxycodone may interact at least in part with DOP-KOP receptor heterodimers to produce its *in vivo* pharmacological profile herein that was unique among the seven opioid ligands assessed. In support of this notion, the κ_2-opioid receptor agonist, bremazocine (Romer *et al.*, 1980), failed to inhibit GI transit in the rat (Petrillo *et al.*, 1984). Hence, i.c.v. oxycodone's partial agonism for inhibition of castor oil-induced diarrhoea may be explained by its low affinity agonist activity at the MOP receptor together with its ability to activate DOP-KOP receptor heterodimers.

DPDPE was devoid of constipation herein in agreement with others that DPDPE neither inhibited castor oil-induced diarrhoea nor induced anti-propulsive actions (Porreca *et al.*, 1984; Shook *et al.*, 1989; Massi *et al.*, 1994). Similarly, our findings that U69,593 is devoid of constipation in rats re-capitulates work by others for U69,593 (La Regina *et al.*, 1988) and U50,488H in rats (Burks *et al.*, 1988). For respiratory depression, we found that both i.c.v. DPDPE and U69,593 were inactive in agreement with others for DOP (Porreca *et al.*, 1984; Shook *et al.*, 1989; Broccardo and Improta, 1992; Massi *et al.*, 1994) and KOP receptor agonists (Lahti *et al.*, 1985; La Regina *et al.*, 1988; Butelman *et al.*, 1993; Riviere, 2004; Mutolo *et al.*, 2007). U69,593's lack of respiratory depressant effects may be due to the fact that in the rodent CNS, U69,593 inhibits pre-synaptic release of the neurotransmitters, glutamate and GABA (Hjelmstad and Fields, 2003) that have opposing actions on respiratory function in rodents (Yamada *et al.*, 1981; Greer *et al.*, 1991).

Insight into the cellular mechanisms underpinning between-opioid differences in the antinociception, constipation and respiratory depression profiles in rats described here

comes from *in vitro* data showing that opioid ligands differentially recruit G-proteins vis-à-vis β-arrestin-2 at the MOP receptor (McPherson *et al.*, 2010; Molinari *et al.*, 2010; Kelly, 2013) and/or the DOP receptor (Molinari *et al.*, 2010). Although fentanyl and morphine have similar potencies and efficacies for G-protein interactions at MOP and DOP receptors, morphine is a partial agonist, whereas fentanyl is a full agonist for β-arrestin-2 recruitment at the MOP receptor (Molinari *et al.*, 2010). Conversely for β-arrestin-2 recruitment at the DOP receptor, fentanyl is a partial agonist, whereas morphine is a full antagonist (Molinari *et al.*, 2010). For G-protein recruitment at the MOP, buprenorphine has ~4.5-fold higher efficacy compared with the DOP receptor, whereas it lacks efficacy for β-arrestin-2 recruitment at both MOP and DOP receptors (Molinari *et al.*, 2010). In the GTP-γS assay in cells expressing cloned MOP, DOP or KOP receptors, oxycodone was a full MOP receptor agonist, whereas it was inactive at the DOP or KOP receptor (Lalovic *et al.*, 2006; McPherson *et al.*, 2010). By contrast, oxycodone was a partial agonist for β-arrestin-2 recruitment at the MOP receptor (McPherson *et al.*, 2010; Kelly, 2013). Further work beyond the scope of that described here is clearly required to gain deeper insight into specific pathway-dependent agonist and antagonist efficacy and signalling at the MOP receptor.

Conclusion

Our findings have shown differences between antinociception, constipation and respiratory depression in rats administered i.c.v. bolus doses of one of seven opioid agonists tested, such that no two opioids had the same profile. Regardless of the precise mechanisms, our *in vivo* findings, together with other recent work from our laboratory (Varamini *et al.*, 2012), suggest that these three pharmacodynamic responses are differentially regulated and that it may be possible to discover novel strong opioid analgesics with markedly improved adverse event profiles.

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Conflict of interest

None.

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