Effects of oxycodone and diazepam alone and in combination on operant nociception

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Developing effective analgesics with fewer unwanted side effects is a pressing concern. Due to a lack of effective nonopioid options currently available, an alternative approach termed *opioid-sparing* evaluates the ability of a coadministered drug to reduce the amount of opioid needed to produce an antinociceptive effect. Opioids and benzodiazepines are often coprescribed. Although this approach is theoretically rational given the prevalent comorbidity of chronic pain and anxiety, it also has inherent risks of respiratory depression, which is likely responsible for the substantial percentage of fatal opioid overdoses that have involved benzodiazepines. Moreover, there have been no clinical trials to support the effectiveness of this drug combination nor has there been corroborative preclinical evidence using traditional animal models of nociception. The present studies examined the prescription μ-opioid analgesic oxycodone (0.003–0.1 mg/kg) and the prototypical benzodiazepine anxiolytic diazepam (0.03–1.0 mg/kg), alone and in combination, using an animal model of pain that examines the restoration of conflict-related operant behavior as evidence of analgesia. Results documented significant dose-related increases in thermal threshold following oxycodone treatment. Diazepam treatment alone did not produce significant antinociception. In combination, diazepam pretreatment shifted oxycodone functions upward in a dose-dependent manner, but the additive effects were limited to a narrow dose range. In addition, combinations of diazepam and oxycodone at higher doses abolished responding. Taken together, though intriguing, these findings do not provide sufficient evidence that coadministration of an anxiolytic will result in clinically relevant opioid-sparing for pain management, especially when considering the inherent risks of this drug class combination. ***Behavioural Pharmacology*** **31: 168–173**

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Introduction

The need for effective analgesics with fewer unwanted side effects, such as abuse potential, is a well publicized concern (Corbett *et al.*, 2006; Christie *et al.*, 2017). However, despite highly active laboratory and clinical research efforts over the last 50 years, there have been limited successes in the development of new forms of analgesic drugs (Kissin, 2010). As the search continues for nonopioid analgesics, an alternative tactic termed *opioid-sparing* has been explored in which an opioid is combined with another drug in an attempt to minimize the opioid dose necessary to produce comparable antinociception (Gilron *et al.*, 2013; Nielsen *et al.*, 2017).

Two drug classes that are already commonly coprescribed to patients suffering from acute and chronic painful conditions, especially musculoskeletal pain, are opioids and benzodiazepines (Larochelle *et al.*, 2015). This approach is theoretically rational because, in addition to muscle relaxation, benzodiazepines produce anxiolytic effects that may diminish the anxiety that accompanies clinical pain. Indeed, researchers have long argued that anxiety and pain are closely intertwined (Hill *et al.*, 1954; Dews, 1974; Von Korff and Simon, 1996). Importantly, however, the combination of opioids and benzodiazepines also poses considerable risk in which both drug classes depress ventilation through differing mechanisms and, depending on doses, can induce fatal respiratory depression (Gerak *et al.*, 1998; Gueye *et al.*, 2002; Nielsen and Taylor, 2005). Thus, it is unsurprising that the rate of emergency room visits and death associated with their co-use has been increasing (Jones and McAninch, 2015; Sun *et al.*, 2017). Indeed, the Centers for Disease Control has reported that 31% of fatal opioid overdoses in recent years involved benzodiazepines (Chen *et al.*, 2014). Despite these risks, coprescription of opioids and benzodiazepines has increased during the past decade (Hwang *et al.*, 2016). These ominous trends led the Food and Drug Administration in 2016 to issue boxed warnings to inform healthcare providers and patients of the serious risks associated with the combined use of benzodiazepines and opioids.

Surprisingly, the practice of coprescribing opioids and benzodiazepines developed in the absence of any clinical trials demonstrating benefit over the prescription of opioids alone (Kim *et al.*, 2017). In addition, most studies using animal models of nociception do not support the idea that conventional benzodiazepines can improve

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opioid analgesia. First, benzodiazepines are not generally considered to be effective analgesics, a conclusion that is supported by their lack of antinociception efficacy in rodent tail flick assays (Eisenberg, 1985; Wang *et al.*, 1995; however, see Zambotti *et al.*, 1991, for small effects of diazepam). Second, coadministering a benzodiazepine like diazepam with an opioid has also failed to potentiate opioid antinociception in a variety of animal models of pain. For example, Abbott and Franklin (1986) showed that pretreatment with diazepam did not alter morphine antinociception in a thermal assay in the rat (tail flick) and, interestingly, insurmountably blocked morphine antinociception in a chemical assay (formalin test). More recently, Gonek *et al.* (2017) demonstrated in mice using another thermal assay (warm water tail immersion) that although diazepam had an intriguing ability to reduce tolerance to the antinociceptive effects of both oxycodone and hydrocodone, it failed to potentiate the acute antinociceptive effects of either opioid.

The failures to enhance opioid antinociception following diazepam treatment highlighted above may be attributable to its lack of analgesic efficacy but, alternatively, could reflect constraints of assays used to measure antinociception. That is, most animal models of pain, including the tail flick, formalin test, and warm water tail immersion, rely on reflexive or other unconditioned behavior to assess antinociception. However, research using functional imaging has made it increasingly clear that the experience of pain has high heterogeneity (Moisset and Bouhassira, 2007; Martucci and Mackey, 2016). Therefore, preclinical assessments of analgesic action may benefit from examining diverse nociceptive conditions. In the present experiments, an operant nociception assay for nonhuman primates (Kangas and Bergman, 2014) was used to assess the ability of benzodiazepines to enhance opioid analgesia. Unlike most animal models of pain that assay simple spinal reflexes or unconditioned behavioral reactions, operant-based procedures can be used to assess the ability of drug treatment to restore volitional behavioral responses previously suppressed by nociceptive stimuli (Morgan *et al.*, 2008; Negus, 2019; Withey *et al.*, 2020). Conceivably, benzodiazepines may aid analgesia under these conditions through its anxiolytic actions on pain-suppressed behavior. We first assessed the antinociceptive effects of the prescription μ-opioid analgesic oxycodone. Next, the effects of the prototypical benzodiazepine anxiolytic diazepam were studied alone and in combination with oxycodone.

Method

Subjects

Six adult male squirrel monkeys (*Saimiri sciureus*) were used in the present studies. The subjects had served previously in studies of dopamine-related drugs or cannabinoids, but had not received drug treatment for at least 6 months prior to the present studies. In addition, none of the subjects had previous experience with opioids, benzodiazepines, or nociception assays. Subjects were individually housed in a temperature- and humidity-controlled vivarium with a 12-hour light/dark cycle (7 a.m./7 p.m.). Subjects had unlimited access to water in the home cage and were maintained at approximate free-feeding weights by postsession access to a nutritionally balanced diet of high protein banana-flavored biscuits (Purina Monkey Chow, St. Louis, Missouri, USA). In addition, fresh fruit and environmental enrichment were provided daily. Experimental sessions were conducted 5 days a week (Monday to Friday). The experimental protocol for the present studies was approved by the Institutional Animal Care and Use Committee at McLean Hospital. Subjects were maintained in a facility licensed by the US Department of Agriculture and in accordance with guidelines provided by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, Commission on Life Sciences, National Research Council (2011).

Apparatus

Details and schematics of the operant nociception chamber can be found in Kangas and Bergman (2014). Subjects sat in a custom-built Plexiglas chair measuring 25 × 25 × 40 cm housed in a 50 × 50 × 75 cm sound- and light-attenuating enclosure. A digital video camera was mounted in the inside upper-right corner of the enclosure for real-time session monitoring and an infusion pump (PHM-100-10; Med Associates, St. Albans, Vermont, USA) was mounted outside the left wall of the enclosure for the delivery of liquid reinforcement. Each operation of the pump delivered 0.15 ml of 30% sweetened condensed milk (70% water) into an easily accessible shallow well (2.5 cm diameter) of a custom-designed Plexiglas fluid dispenser (5 × 3.5 × 1.27 cm) mounted to the inside front wall of the chair. Previous studies in our laboratory have found that a small volume (0.15 ml) of this liquid serves as a powerful reinforcer for squirrel monkeys that is very resistant to satiation even under free-feeding conditions (Kangas *et al.*, 2016). Three horizontally arrayed white stimulus lights (2.5 cm in diameter) were mounted 50 cm above the enclosure floor, spaced 10 cm apart and centered above the fluid dispenser. A telegraph key was secured to a shelf 15 cm above the stimulus lights, and a custom-built stainless steel 500w/120v thermode (1.27 cm diameter; 15.24 cm length) with fiberglass leads hung from the telegraph key button via a 5 cm chain. A downward pull of the thermode closed the telegraph key circuit, making an electrical contact that could serve as a response. A temperature sensor (TBC-72.OG; Convectronics, Haverhill, Massachusetts, USA) was attached to the upper end of the thermode which also was attached via the fiberglass leads to a 120v, 15amp temperature control unit (Control Console 006-12015; Convectronics). This unit served as a thermostat

and controlled the temperature of the thermode with a resolution of ±1°C. All temperature settings and adjustments were made by the experimenter. Other experimental events (i.e., pull detection, operation of stimulus lights, milk delivery) and data collection were controlled by Med Associates interfacing equipment and operating software.

Procedure

Thermal threshold tests

Subjects were seated in the chair and first trained to complete 3 seconds duration pull responses on the thermode (see Kangas and Bergman, 2014, for training details). Next, thermal thresholds were determined during sessions in which subjects were exposed to an ascending sequence of thermode temperatures. Each temperature was evaluated during a five-trial block, and each trial began with the illumination of left and right stimulus lights. Completion of a 3-second pull terminated the trial, extinguished all stimulus lights within the chamber, delivered 0.15 ml of milk into the well, and initiated the 10-second intertrial interval. Each five-trial block was followed by a 2-minute blackout period during which all stimulus lights in the chamber were off and the thermode temperature was increased. An ascending sequence of thermal stimulation was used in which the thermode was initially 38°C (approximate body temperature) for the first five trials. Following completion of the five trials, the thermode temperature increased 2°C during the 2-minute blackout and remained at 40°C for the next five trials (i.e., 2°C step-size). If all five trials were completed, the thermode was again increased by 2°C during the next 2-minute blackout period. Trials continued until 20 seconds elapsed without a successful response (i.e., limited hold 20 seconds), which resulted in session termination. Blocks of five trials at each temperature were used to provide repeated assessment of the subject's performance at that temperature. Thermal thresholds served as the primary dependent measure and were defined as the highest temperature at which the subject completed at least three of the five trials in a block prior to session termination. An imposed maximum thermode temperature of 60°C was in effect throughout all studies to preclude contact that might produce tissue damage. To ensure that thermal thresholds were a function of temperature (not number of trials into a session), determinations were conducted from different temperature start points with the proviso that it be at least 2, and no more than 10, steps (i.e., 4°C) below the expected thermal threshold. Thermal threshold tests under a 3-second pull duration were conducted at least five times in each subject in this manner, that is, using varying start points prior to drug administration.

Drug effects on thermal thresholds

The effects of intramuscular (i.m.) injections of saline and a range of doses of the prescription μ-opioid agonist oxycodone (0.003–0.1 mg/kg) and the benzodiazepine diazepam (0.03–1.0 mg/kg) on thermal threshold values were examined. Drug combination studies also were conducted to assess additive antinociceptive potential of cumulative oxycodone doses following treatment of 0.03 and 0.1 mg/kg diazepam. For each drug, conventional cumulative dosing procedures (Spealman, 1985; Kangas and Bergman, 2014) were used to permit the determination of the effects of incremental i.m. doses of oxycodone or diazepam in a single test session. Cumulative doses were administered at the beginning of sequential 10-minute timeout periods that preceded repeated threshold determinations. During cumulative dosing procedures, temperature start points were 2–4 steps below the subject's threshold following saline treatment. To examine the effects of drug combinations, 0.03 or 0.1 mg/kg diazepam was administered immediately prior to an oxycodone cumulative dosing session. Doses of each drug were studied up to those that eliminated responding. Each cumulative dose determination was conducted after at least three intervening days in which thermal control sessions were conducted and baseline thermal threshold values re-established.

Drugs

Oxycodone and diazepam were purchased from Sigma Pharmaceuticals (St. Louis, Missouri, USA). Oxycodone was prepared in a 0.9% saline solution. Diazepam was prepared in a 20:20:60 mixture by volume of 95% ethanol, Tween-80, and 0.9% saline. All drug solutions were refrigerated and protected from light. During test sessions, saline or doses of drug were administered in volumes of 0.3 ml/kg body weight or less by i.m. injection into calf or thigh muscle. Drug concentrations are expressed in terms of their free base.

Data analysis

Thermal threshold served as the primary dependent measure of nociception and was defined as the highest temperature at which the subject completed at least three of the five trials. Data from drug tests were first normalized for individual subjects by assessing changes from individual subject threshold values across dose determinations, and then presented as group means of those changes in threshold. A one-way repeated measures analysis of variance (ANOVA) was conducted to evaluate the statistical significance of each drug treatment alone, relative to thermal thresholds observed following saline treatment. To evaluate the effects of diazepam pretreatment on oxycodone antinociception, a two-way repeated measures ANOVA (diazepam dose × oxycodone dose) was conducted with coadministered oxycodone (0, 0.003, or 0.01 mg/kg) and diazepam (0, 0.03, or 0.1 mg/kg) as factors. When appropriate, ANOVAs were followed by a Dunnett's Multiple Comparison Test to evaluate the statistical significance of thermal threshold increases over individual normalized control values. The criterion for

significance was set at $P < 0.05$. All statistical analyses were conducted using GraphPad Prism 8 Software (San Diego, California, USA).

Results

Thermal threshold tests

At least five thermal threshold determinations were conducted with each subject and the reliability of threshold values across repeated determinations was remarkably high for each subject. Baseline thermal thresholds did not deviate from modal values by more than one 2°C step size in five of six subjects tested. In addition, saline treatment, whether administered prior to a single thermal threshold determination or across as many as four consecutive determinations, produced no systematic changes in thermal thresholds. These low levels of variability in control thermal thresholds and following saline treatment were similar to those observed in previous studies (Kangas and Bergman, 2014).

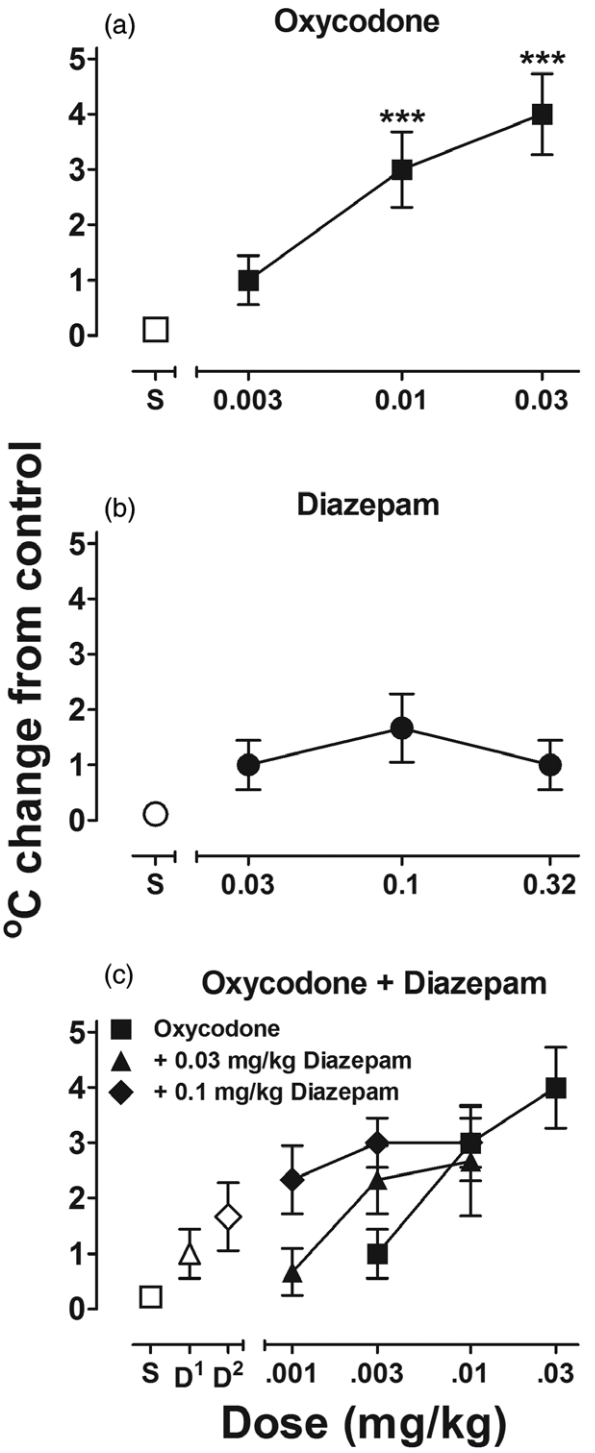
Drug effects on thermal thresholds

Figure 1 shows dose response functions for oxycodone and diazepam tested alone and in combination. Oxycodone produced significant [$F(3,15) = 16.92$, $P < 0.0001$] dose-related increases in thermal threshold values (Fig. 1a). A small increase of approximately 1°C in the mean threshold value was evident after the lowest dose of oxycodone tested (0.003 mg/kg), whereas significant increases of approximately 3 and 4°C in thermal threshold values were observed following, respectively, 0.01 mg/kg ($P < 0.001$) and 0.03 mg/kg ($P < 0.001$) oxycodone. The highest cumulative dose of oxycodone tested, 0.1 mg/kg, abolished responding in five of six subjects. A 6°C increase from control values was observed in the subject that responded following administration of 0.1 mg/kg oxycodone.

Diazepam (Fig. 1b) produced a small elevation in group average thermal threshold values (~1–2°C) across a range of doses (0.03–0.32 mg/kg). A modest peak in group average thermal threshold of approximately 2°C was observed following the cumulative dose of 0.1 mg/kg diazepam. However, the increases in thermal thresholds following treatment with 0.03–0.32 mg/kg diazepam were not statistically different than thermal thresholds following saline treatment [$F(3,15) = 3.06$, $P = 0.06$]. The highest dose of diazepam tested (1.0 mg/kg) abolished responding in five of six subjects and produced a 2°C change from control threshold values in the remaining subject.

Figure 1c shows dose-response functions for oxycodone alone (squares) and following a 0.03 mg/kg (triangles) and 0.1 mg/kg (diamonds) pretreatment of diazepam. Diazepam pretreatment dose-dependently shifted the oxycodone cumulative dose-response function upward. However, unlike following treatment with oxycodone

Fig. 1



Group mean (±SEM) thermal threshold changes from baseline following saline (S) and cumulative doses of oxycodone (a), diazepam (b), oxycodone alone (squares) and following treatment of 0.03 mg/kg diazepam (triangles) and 0.1 mg/kg diazepam (diamonds) (c). In (c), (D^1) indicates values of 0.03 mg/kg diazepam alone, (D^2) indicates values of 0.1 mg/kg diazepam alone. $n = 6$, ***$P < 0.001$.

alone, responding was abolished in all subjects following administration of 0.03 mg/kg oxycodone during both diazepam pretreatment conditions. Because the dose of 0.001 mg/kg oxycodone was not examined alone and, as well, the dose of 0.03 mg/kg oxycodone abolished performance when paired with diazepam, a two-way ANOVA for oxycodone and diazepam treatment was limited to only the doses of each drug that were examined both alone and in combination (i.e., 0.03–0.1 mg/kg diazepam and 0.003–0.01 oxycodone). This truncated analysis revealed main effects of both oxycodone [$F(2,10) = 11.14$, $P < 0.01$] and diazepam [$F(2,10) = 5.05$, $P < 0.05$] administration on thermal threshold. However, although both drugs contributed to the antinociceptive effects observed under this limited range of drug combinations, no significant interaction was observed [$F(4,20) = 1.24$, $P = 0.33$].

Discussion

The prescription μ-opioid analgesic oxycodone produced significant dose-related increases in thermal threshold. These findings systematically replicate thermal threshold increases observed following treatment with other opioid analgesics under similar operant nociception conditions (Kangas and Bergman, 2014). Consistent with data from previous studies using traditional animal models of pain, the prototypical benzodiazepine anxiolytic diazepam did not produce significant group-mean changes in thermal thresholds across a range of doses. Pretreatment with 0.03 and 0.1 mg/kg diazepam was able to modestly shift the oxycodone function upward in a dose-dependent manner. However, when combining either dose of diazepam tested with the maximally effective dose of 0.03 mg/kg oxycodone, responding was abolished in all subjects. When coadministered at lower doses, the main effects of oxycodone and diazepam were significant, but the interaction between oxycodone and diazepam was not, suggesting that the combination of these drugs produced an additive antinociceptive effect. Although the antinociceptive properties of diazepam are clearly modest, these effects may be relevant in certain clinical contexts where low doses of oxycodone are otherwise effective. Indeed, the upward shifts were limited to the lower limb of the oxycodone dose-response function (0.001–0.003 mg/kg) and were negligible at higher doses (0.01 mg/kg). It should be noted, however, that the inability to examine diazepam across a broader range of oxycodone doses in this assay prevents a more comprehensive characterization of diazepam's adjuvant potential.

One may view the escalating thermal conditions arranged in the present study as a variant of a conflict procedure. In prototypical conflict procedures, operant responding maintained by positive reinforcement is suppressed (punished) in certain trial types but not others, using stimuli such as electric shock (Geller and Seifter, 1960; Vogel *et al.*, 1971). In the present study, thermal stimuli suppressed responding following sufficiently high operandum temperatures which, as shown here and previously, can be surmounted with analgesic treatment. However, several benzodiazepines, including diazepam, have well-known anticonflict effects that have been implicated in the attenuation of anxiety-related behavior (Sepinwall *et al.*, 1978; Rowlett *et al.*, 2006). There is an increasing understanding in analgesia research that the report of pain is a multimodal response that may reflect anxiety as well as nociception. From this perspective, the ability of diazepam – which failed to demonstrate significant antinociceptive efficacy alone – but was able to produce modest enhancement of oxycodone's antinociceptive effect, perhaps can be attributed to its anticonflict (antipunishment) actions.

Taken together, the present findings do not provide sufficient evidence that pairing an anxiolytic with an opioid analgesic will result in meaningful opioid-sparing for pain management. Moreover, as discussed above, coadministration of opioids and benzodiazepines have inherent dangers of aggravated respiratory depression, evident in laboratory findings and verified by the epidemiology of opioid overdose. In addition, given the relatively high abuse potential of both oxycodone (Wightman *et al.*, 2012) and diazepam (Griffiths and Weerts, 1997; O'Brien, 2005), a drug combination of the two is unlikely to be a viable candidate analgesic outside of highly controlled clinical settings (Jones *et al.*, 2012), especially in individuals with a history of substance use disorder (O'Brien *et al.*, 2017). Finally, the present data suggest that this drug combination may produce substantial behavioral disruption under more severe nociceptive conditions in which high doses of an opioid are required. In conclusion, although the present data provide intriguing diazepam-related upward shifts in the oxycodone antinociception dose-response function, the shifts are modest, maximal effects are not elevated, and response disruption was produced at higher dose combinations. Therefore, these studies provide insufficient evidence that treatment with an anxiolytic result in smaller opioid dosing requirements for clinically-relevant antinociception.

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Conflicts of interest

There are no conflicts of interest.

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