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Research report

Reversal of oxycodone and hydrocodone tolerance by diazepam

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ABSTRACT

The Centers for Disease Control has declared opioid abuse to be an epidemic. Overdose deaths are largely assumed to be the result of excessive opioid consumption. In many of these cases, however, opioid abusers are often polydrug abusers. Benzodiazepines are one of the most commonly co-abused substances and pose a significant risk to opioid users. In 2016, the FDA required boxed warnings – the FDA's strongest warning – for prescription opioid analgesics and benzodiazepines about the serious risks associated with using these medications at the same time. The point of our studies was to evaluate the interactions between these two classes of drugs. We investigated whether diazepam adds to the depressant effects of opioids or do they alter the levels of tolerance to opioids. In the present study, we have found that the antinociceptive tolerance that developed to repeated administration of oxycodone was reversed by an acute dose of diazepam. Antinociceptive tolerance to hydrocodone was also reversed by acute injection of diazepam; however, a fourfold higher dose of diazepam was required when compared to reversal of oxycodone-induced tolerance. These doses of diazepam did not potentiate the acute antinociceptive effect of either opioid. The same dose of diazepam that reversed oxycodone antinociceptive tolerance also reversed oxycodone locomotor tolerance while having no potentiating effects. These studies show that diazepam does not potentiate the acute effect of prescription opioids but reverses the tolerance developed after chronic administration of the drugs.

1. Introduction

Reducing opioid overdose deaths is an important public health and drug policy goal. Much of the current opioid epidemic can be attributed to the rise in use of prescription opioid pain analgesics such as oxycodone and hydrocodone. These drugs have become widely prescribed, with enough opioid analgesics sold in 2010 to medicate every American adult with a typical dose every 4 h for 1 month. The CDC reported that in the United States, at least half of all opioid overdose deaths involve a prescription opioid (Paulozzi et al., 2011).

The dangers of accidental opioid overdose are mainly due to respiratory depressive effects. Chronic use of opioids results in the development of tolerance (a decrease in pharmacologic response following repeated or prolonged drug administration) to the analgesic, euphoric, and respiratory depressive effects. This leads to addicts and patients taking higher doses in order to obtain the euphoric high or the analgesic effects, respectively. However, it has been shown that tolerance to different effects of opioids do not occur at the same rate or to the same extent (Hill et al., 2016). It has been suggested that, in man, tolerance to euphoria develops to a greater extent than to respiratory depression (White and Irvine, 1999).

Opioid overdose deaths are largely assumed to result from excessive opioid administration alone. However, opioid abusers are often polydrug users, consuming benzodiazepines, ethanol, cocaine and/or gabapentoids along with opioid drugs. Benzodiazepines and ethanol have been found to pose a significant risk to chronic opioid users, particularly in those taking methadone (National Treatment Agency and for Substance Misuse [NTA], 2007). The CDC has reported that benzodiazepines were involved in 31% of opioid related drug poisoning deaths in recent years (Chen et al., 2014). Benzodiazepines, ethanol, and opioids are all considered central nervous system depressants and their effects may be additive or synergistic. Recently, our lab has published that low doses of ethanol and diazepam, which have no observable effect of their own, significantly and dose-dependently reduced the antinociceptive tolerance produced by morphine while not affecting the acute responses (Hull et al., 2013). Low doses of ethanol reversed morphine tolerance at the level of single brain neu-

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rons (Llorente et al., 2013) and in a rodent model of respiratory depression (Hill et al., 2016).

A major limitation of the previously described studies is that they have not investigated oxycodone and hydrocodone, two commonly prescribed opioid analgesics. Limited research has been done with these drugs when compared to the opioid standard, morphine, and the illicit compound heroin. Although all are considered opioids, these compounds may differently interact through the μ opioid receptor (MOR). They have been shown to have different pharmacokinetic properties, varying affinities for the μ opioid receptor, potentially interact with other opioid receptors and have different off-target effects (Nielsen et al., 2007). Therefore, it is important to investigate how commonly prescribed opioids compare in their effects to morphine.

The goals of this study were to determine if benzodiazepines potentiate the acute antinociceptive effects of commonly prescribed opioids as well as to determine if they act to reduce tolerance to these opioids. We characterized the development of antinociceptive tolerance to oxycodone and hydrocodone in mice and investigated whether diazepam could reverse tolerance as it does morphine-induced tolerance. Antinociception, as measured by the rodent warm water tail-immersion assay, was chosen for these studies because it has been shown to be a good predictor of antinociception and tolerance for a wide range of compounds in humans. Furthermore, our group has used this assay to extensively investigate the mechanisms of opioid tolerance (Hull et al., 2013, 2010; Smith et al., 2007).

2. Results

2.1. Effects of acute diazepam in drug-naïve mice

Diazepam was administered at doses of 0.5, 1, and 2 mg/kg i.p. and mice were monitored over a 3-h period for behavioral changes and assessed in the warm-water tail immersion test at 30-min intervals over 2 h. No antinociceptive effects or behavioral changes, including locomotor activity, were observed at any of these doses.

2.2. Tolerance development to oxycodone

Baseline latencies were taken prior to the beginning of the hourly subcutaneous injections. Mice were randomly assigned to either a chronic saline or chronic opioid schedule whereby seven hourly injections of isotonic saline or an ED_{80} dose of oxycodone were given s.c. After seven injections, mice were injected with a challenge dose of oxycodone (0.25, 0.5, 1, 2 or 4 mg/kg) at the 8-h time point (Fig. 1). Dose-response curves for oxycodone after chronic injections of saline generated similar ED_{50} values to those in acute dose response experiments (1.19 mg/kg (1.00–1.41, 95% CL)). The ED_{50} was significantly shifted to the right 1.6-fold, indicating tolerance was observed, in the animals chronically injected with oxycodone prior to receiving the challenge injections (1.84 mg/kg (1.58–2.14, 95% CL)). The sample size of each group was 20 animals [Acute Oxycodone, n = 20; Chronic Oxycodone, n = 20].

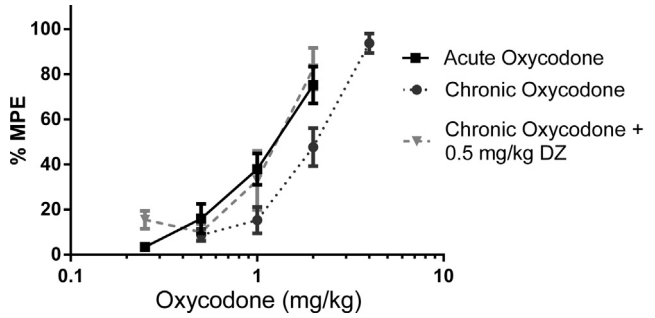

Fig. 1. Tolerance to oxycodone, developed using a single-day injection paradigm, was significantly reversed by 0.5 mg/kg diazepam (n = 10–20). Latency to tail withdrawal (% MPE ± SEM) among mice that were drug naïve, repeatedly treated with oxycodone, or repeatedly treated with oxycodone and diazepam pretreatment 30 min before testing. Various doses of the oxycodone were used for construction of dose-response curves for calculation of ED50 values.

2.3. Reversal of oxycodone antinociceptive tolerance with diazepam in tail immersion assay

Baseline latencies were obtained in the tail immersion test in the morning before any injections. Following the development of tolerance (single day tolerance model), diazepam (0.5 mg/kg i.p.) was administered. Thirty minutes later, the mice were challenged with doses of oxycodone s.c. for construction of dose-response curves for calculation of the ED_{50} values (Fig. 1; Table 1). Diazepam fully reversed the oxycodone-induced tolerance. The sample size of each group was between 10 and 20 animals [Acute Oxycodone, n = 20; Chronic Oxycodone, n = 20; Chronic Oxycodone + 0.5 mg/kg DZ, n = 12]. The same dose of diazepam did not potentiate the antinociception produced by acute doses of oxycodone in naive mice (Fig. 2) [Acute Oxycodone, n = 20; Acute Oxycodone + 0.5 mg/kg DZ, n = 11].

2.4. Tolerance development to hydrocodone

Baseline latencies were taken prior to the beginning of hourly subcutaneous injections. Mice were assigned randomly to either a chronic saline or chronic opioid schedule whereby seven hourly injections of isotonic saline or an ED_{80} dose of hydrocodone were given s.c. After seven injections, all mice were injected with final challenge doses of hydrocodone (1, 2, 4, 8, 16 or 32 mg/kg) at the 8-h time point (Fig. 3). Dose response curves of hydrocodone after chronic injections of saline generated similar ED_{50} values to those in acute dose response experiments (5.51 mg/kg (4.97–6.12, 95% CL)). The ED_{50} was significantly shifted 2.4-fold to the right in the animals chronically injected with hydrocodone prior to receiving the challenge injections (13.18 mg/kg (11.00–15.80, 95% CL)). The sample size of each group was between 6 and 12 animals [Acute Hydrocodone, n = 6; Chronic Hydrocodone, n = 12].

2.5. Reversal of hydrocodone antinociceptive tolerance with diazepam in tail immersion assay

Baseline latencies were obtained in the tail immersion test in the morning before any injections. Following the development of tolerance (single day tolerance model), diazepam was administered i.p. Thirty minutes later, the mice were challenged with doses of hydrocodone s.c. for construction of dose-response curves for calculation of the ED_{50} values (Table 2). In contrast with oxycodone, 0.5 mg/kg diazepam did not fully reverse antinociceptive tolerance to hydrocodone (Fig. 3). 2 mg/kg diazepam fully reversed hydrocodone tolerance and actually significantly potentiated the antinociceptive effect of hydrocodone after chronic administration

Table 1
Diazepam reversal of oxycodone tolerance.

Treatment	Oxycodone ED_{50} (mg/kg (95% C.L.))
Acute Oxycodone + Vehicle	1.19 (1.00–1.41)
Acute Oxycodone + Diazepam (0.5 mg/kg)	1.25 (1.04–1.50)
Chronic Oxycodone + Vehicle	1.84 (1.58–2.14)*
Chronic Oxycodone + Diazepam (0.5 mg/kg)	1.12 (0.88–1.43)

* Significantly different than Acute Oxycodone + Vehicle group based on non-overlapping 95%.