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Acetaminophen modulation of hydrocodone reward in rats

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ABSTRACT

Abuse of prescription opioid analgesics in non-medical context has been on the rise over the past decade. The most commonly abused analgesic in this drug class consists of a combined formulation of hydrocodone and acetaminophen. The present study was aimed to determine the rewarding effects of hydrocodone, acetaminophen, and their combination using the conditioned place preference (CPP) paradigm. Using a 6-day CPP paradigm, rats were paired with hydrocodone (0.5, 1.0 or 5.0 mg/kg) or acetaminophen (50, 100 or 300 mg/kg) to determine whether the drugs given alone would produce a CPP. Rats conditioned with the highest dose of hydrocodone exhibited place preference, whereas rats conditioned with acetaminophen did not demonstrate place preference. In a second experiment, varying doses of hydrocodone and acetaminophen were combined to determine whether acetaminophen would enhance hydrocodone reward. Acetaminophen (100 mg/kg) enhanced the rewarding effects of hydrocodone (1 mg/kg), although the effect was unique to this particular dose combination. Higher or lower doses of acetaminophen combined with hydrocodone did not alter hydrocodone CPP. The present findings suggest that acetaminophen has a limited potential of modulating the rewarding properties of hydrocodone in rats.

1. Introduction

Prescription opioid analgesics are commonly used for the treatment of various acute and chronic pain conditions. Along with the large number of prescriptions for such medications, their non-medical abuse has also been on the rise. The prevalence rate of illicit abuse of prescription opioid analgesics has surpassed that of cocaine, hallucinogens and inhalants (SAMHSA, 2008a). In addition, epidemiological findings further suggest that the illicit use of prescription opioids has led to an increase in emergency room admissions and admission to drug abuse treatment centers (Johnston et al., 2008; SAMHSA, 2008b). In 2007, the National Survey on Drug Use and Health reported that approximately 5% of Americans over the age of 12 had used prescription opioid analgesics in an illicit or non-medical context over the previous 12 months. These findings demonstrate the severity of the situation and highlight the urgency to further study and understand the reasons for the rise in illicit use of opioid analgesics. Studies indicate that the rise in illicit use of prescription opioid analgesics is associated to an increase in the ease of access to such drugs (Dasgupta et al., 2006; Gilson et al., 2004; Katz et al., 2007). Although this suggestion certainly holds true, it does not rule-out other contributing factors.

Many prescription opioid analgesics are cocktail formulations of two compounds, an opioid agonist and a non-narcotic analgesic, such as the combination of hydrocodone and acetaminophen (Vicodin®). This analgesic formulation has gained tremendous popularity and was ranked the most prescribed generic drug in the United States, with over 121 million prescriptions filled in 2008 (SDI/Verispan, 2008). Hydrocodone is a semisynthetic codeine analog, acting as an opioid receptor agonist with approximately equal analgesic and drug discriminatory potencies to that of morphine (Meert and Vermeirsch, 2005; Peckham and Traynor, 2006). Although the rewarding properties of morphine have been extensively studied using animal models of drug reward, such as the CPP paradigm (Bardo et al., 1995), the rewarding properties of hydrocodone have not been investigated. Nonetheless, because of its similarity to morphine, hydrocodone is expected to induce place preference in rodents. Acetaminophen (paracetamol) is a non-steroidal analgesic drug with minimal anti-inflammatory effects (Boutaud et al., 2002). The analgesic effects of acetaminophen have been proposed to be through central mechanisms, such as inhibition of prostaglandins, nitric oxide (Anderson, 2008), and most recently inhibition of anandamide reuptake and activation of transient receptor potential vanilloid 1 (TRPV1) receptors by an acetaminophen metabolite (Hogestatt et al., 2005; Mallet et al., 2008; 2010; Ruggieri et al., 2008). Due to the proposed mechanisms of action, especially its ability to inhibit anandamide uptake, it is probable to consider that acetaminophen may be able to alter the rewarding effects of other drugs, such as opioid analgesics when combined. In fact, a low dose of acetaminophen was shown to produce CPP in rats (Abbott and Hellemans, 2000). Such an effect may increase the rewarding effects of hydrocodone, and thus act as a contributing factor in the increasing cases of non-medical abuse of prescription analgesics.

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2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing 300–400 grams were pair-housed in standard Plexiglas® cages and placed on a 12-hour light/dark cycle (lights on at 6 a.m.) with unrestricted access to food and water. All rats were handled for 5 days prior to the start of the experiments. Experiments took place between 9 a.m. and 2 p.m. Animal care and use was in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication 85-23, Bethesda, MD, USA) and approved by the Institutional Animal Care and Use Committee of Western University of Health Sciences.

2.2. Apparatus

The CPP apparatus was a truncated T-maze, consisting of two adjacent conditioning chambers (L:35×W:30×H:55 cm, each chamber) and a small start box (L:19×W:17×H:55 cm). The conditioning chambers possessed distinct visual and tactile cues. One chamber had alternating black and white horizontally striped walls and a metal rod floor, whereas its adjacent conditioning chamber had alternating black and white vertically striped walls and a metal grid (mesh) floor. The start box had gray walls and a smooth floor texture. The start box was located on the side of the apparatus, toward the center point where the two adjacent conditioning chambers share a wall. Thus on preconditioning and postconditioning test days, when the start box door was opened, rats had the option to enter either one of the two conditioning chambers. Once the rat exited the start box, the door of the start box would close and prevent re-entry into the start box. Rats would then be able to travel from one conditioning chamber to the other without traveling through the start box. The tactile and visual cues were chosen to ensure the apparatus to be balanced, where rats would not exhibit a preference to a given conditioning chamber. Locomotor activity was measured in the CPP apparatus. Place preference and locomotor behaviors were digitally recorded and then quantified using Ethovision XT 5.0 (Noldus Information Technology, Leesburg, VA, USA).

2.3. Drugs

Acetaminophen, hydrocodone bitartrate and morphine sulfate pentahydrate were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Due to the hydrophobicity of acetaminophen, all drugs were dissolved in a vehicle containing 7% dimethyl sulfoxide, 7% polyethylene glycol-300 and 86% distilled water (v/v). Drugs were injected through the intraperitoneal route (i.p.) at a volume of 3 ml/kg. In experiments where both hydrocodone and acetaminophen were used, the drugs were combined and administered as a single injection.

2.4. Procedure

A 6-day CPP paradigm was used, consisting of a preconditioning day, 4 conditioning days with one pairing session per day alternating between saline and drug, and a postconditioning test day. On preconditioning day, rats were placed in the start box for 3 min and then permitted to enter the conditioning chambers once the start box door was opened. Upon exiting the start box, the door was closed at which time rats were given 15 min to travel and explore the two conditioning chambers. On conditioning days, rats were injected with saline or drug and placed in one of two conditioning chambers for 30 min. Given the balanced apparatus (see Table 1); drug pairing to a particular compartment was performed in an unbiased arrangement. In experiment 1, drug pairing sessions consisted of administration of hydrocodone (vehicle, 0.5, 1.0 or 5.0 mg/kg) or our positive control morphine (5.0 mg/kg). In experiment 2, rats were conditioned with acetaminophen (vehicle, 50, 100, or 300 mg/kg) or morphine (5.0 mg/kg). In experiment 3, rats were conditioned with vehicle, or a combination of hydrocodone (1.0 or 5.0 mg/kg) and acetaminophen (50, 100 or 300 mg/kg). On the postconditioning test day, rats were placed in the start box for 3 min and then permitted to enter the conditioning chambers. The amount of time spent in the drug-paired or saline-paired chamber was measured for 15 min. Conditioned place preference was defined as greater time spent in the drug-paired chambers on the postconditioning test day between rats conditioned with drug versus rats conditioned with vehicle.

Acute locomotor activity was measured while the rats were placed in the conditioning chamber after the first drug administration. Distance traveled (cm) was measured as a marker of locomotor activity.

Table 1
Mean time spent in seconds (±S.E.M.) of saline treated rats.

	Experiment 1A		Experiment 1B	
	Horizontal stripes	Vertical stripes	Horizontal stripes	Vertical stripes
Preconditioning	436.4 (33.11)	463.7 (33.11)	418.5 (27.85)	481.6 (27.85)
Postconditionig	477.6 (42.64)	422.4 (42.64)	453.5 (59.57)	446.6 (59.57)

Rats did not demonstrate a significant difference in time spent on the horizontally or vertically striped conditioning compartments on preconditioning day or on postconditioning day upon 4 days of saline conditioning.

2.5. Statistical analysis

The amount of time that the rats spent in the drug-paired side on the postconditioning test days across different drug conditioning groups was analyzed using one-way ANOVAs. One-way ANOVA was also used to analyze locomotor activity data with drug treatment as the factor. Post-hoc pair-wise comparisons were made using Dunnett's test to detect statistical differences between vehicle and drug treated groups. All significance was set at the 5% probability level ($p<0.05$). Multiple cohorts of rats were used to collect data for each experiment. Each cohort contributed to $n=2$–3 rats per group.

3. Results

The effect of saline conditioning on determination of a balanced CPP apparatus is shown in Table 1. Rats did not exhibit a significant preference for horizontally or vertically striped conditioning chambers on preconditioning or after 4-days of saline conditioning on postconditioning days, demonstrating the balanced design of the CPP apparatus.

The effects of hydrocodone and acetaminophen pairings on the development of CPP are shown in Fig. 1. Rats conditioned with hydrocodone (5.0 mg/kg) or morphine (5.0 mg/kg) spent more time in the drug-paired chamber as compared to vehicle conditioned rats, drug main effect: $F(4,34)=5.228$, $p<0.01$ (Fig. 1A). On the other hand, rats did not spend more time in the drug-paired chamber for any of the conditioning doses of acetaminophen, but again exhibited more time spent versus vehicle when conditioned with morphine, $F(4, 31)=4.295$, $p<0.01$ (Fig. 1B).

The effects of acetaminophen on hydrocodone CPP are illustrated in Fig. 2. An inverted U-shaped curve was detected when rats were conditioned with varying doses of acetaminophen (50, 100, or 300 mg/kg) combined with hydrocodone (1.0 mg/kg). More specifically, rats conditioned with acetaminophen (100 mg/kg) combined with hydrocodone (1.0 mg/kg) spent a greater amount of time in the drug-paired chamber as compared to rats conditioned with vehicle, $F(4, 41)=3.451$, $p<0.01$ (Fig. 2A). Rats conditioned with lower or higher doses of acetaminophen (50 or 300 mg/kg) combined with hydrocodone (1.0 mg/kg) did not spend more time in the drug-paired chamber as

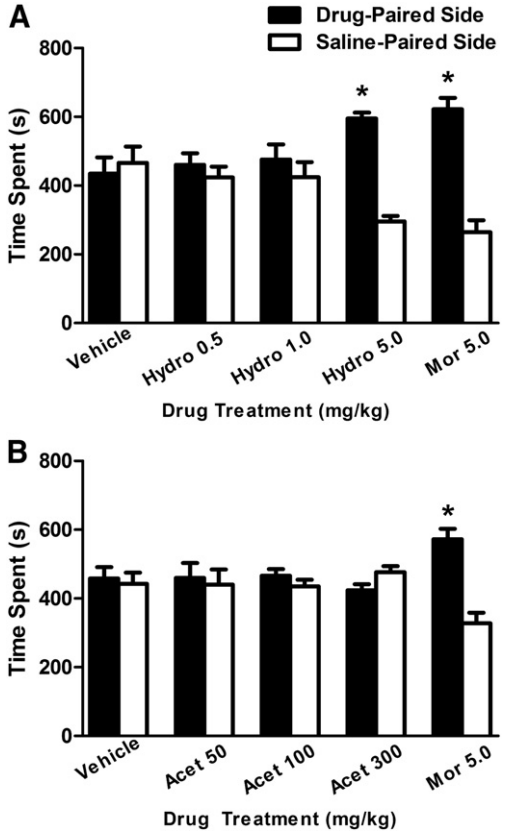

Fig. 1. Time spent in the drug-paired chamber following hydrocodone or acetaminophen pairings in the CPP paradigm. A) The highest dose of hydrocodone (5.0 mg/kg) produced CPP in rats. B) Acetaminophen did not produce CPP at any dose tested. Morphine (5.0 mg/kg), our positive control did produce CPP. *Denotes a significant difference in time spent on the drug-paired side between drug treated versus vehicle treated rats. In panel A, $n = 7$–8 rats per group; in panel B, $n = 6$–8 rats per group.

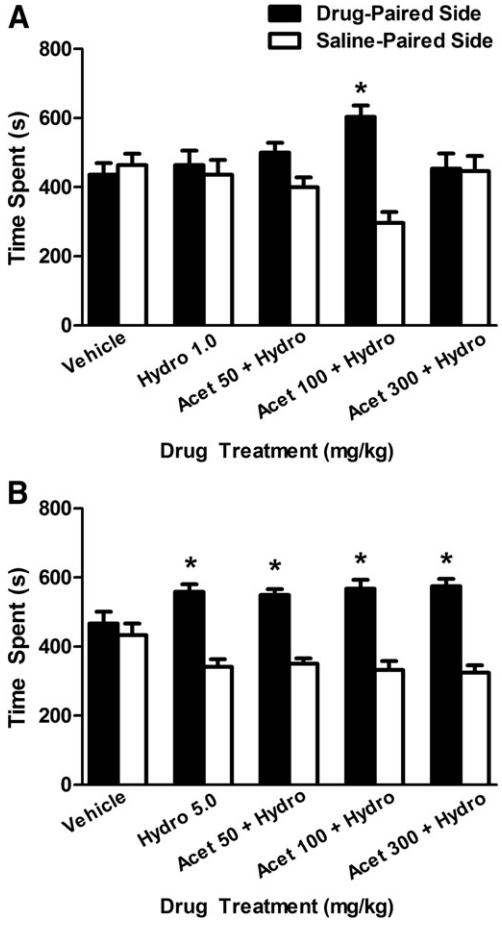

Fig. 2. Effects of acetaminophen on hydrocodone CPP. A) Acetaminophen (100 mg/kg) enhanced the rewarding effects of hydrocodone (1.0 mg/kg), while other acetaminophen doses were ineffective. B) Acetaminophen did not enhance CPP produced by hydrocodone (5.0 mg/kg). *Denotes a significant difference in time spent on the drug-paired side between drug treated versus vehicle treated rats. In panels A and B, $n = 8$–9 rats per group.

compared to the vehicle-conditioned controls. In contrast, all rats that were conditioned with varying doses of acetaminophen (50, 100 or 300 mg/kg) combined with a higher dose of hydrocodone (5.0 mg/kg) spent a greater amount of time in the drug-paired chamber as compared to rats conditioned with vehicle, $F(4,39) = 3.189$, $p < 0.05$ (Fig. 2B).

Locomotor activity data during the first conditioning session is presented in Fig. 3. Rats administered hydrocodone (5.0 mg/kg) alone or combined with acetaminophen (100 mg/kg) exhibited greater distance traveled as compared to rats administered vehicle, $F(5,30) = 4.696$, $p < 0.01$ (Fig. 3). Rats administered acetaminophen (100 mg/kg), hydrocodone (1.0 mg/kg) alone or when combined did not differ in their distance traveled from vehicle treated rats.

4. Discussion

The purpose of the present study was two-fold. First, we sought to determine if hydrocodone or acetaminophen would induce rewarding effects by measuring CPP in rats; and second, whether acetaminophen would alter hydrocodone CPP. Our findings demonstrated for the first time that hydrocodone does possess rewarding properties as it is able to produce CPP in rats. This finding was expected as other opioid agonists such as morphine (McBride et al., 1999; Randall et al., 1998), methadone (Steinpreis et al., 1996) and fentanyl (Finlay et al., 1988) are able to produce CPP. Further, our data indicate that none of the doses of acetaminophen alone produced a CPP in the rats tested. This finding is in contrast to that of Abbott & Hellemans (2000) who showed that a low dose of acetaminophen (50 mg/kg) produced CPP, whereas a higher dose (200 mg/kg) did not produce CPP in rats. The differences in the findings are likely due to several factors, such as strain of rat used (Sprague–Dawley vs. Long–Evans), route of administration (intraperitoneal vs. rectal), and factors related to the CPP paradigm (CPP apparatus, method of drug assignment and conditioning paradigm).

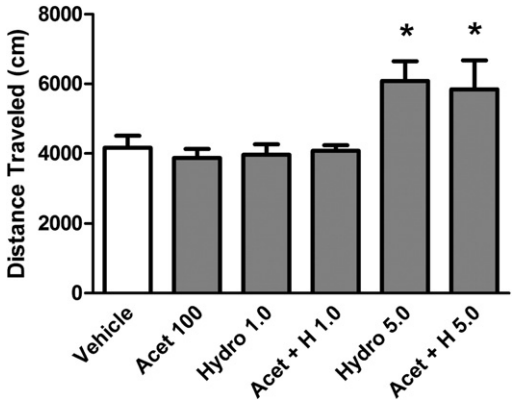

Fig. 3. Effects of acute hydrocodone and acetaminophen on locomotor activity. Rats injected with hydrocodone (5.0 mg/kg) alone or when combined with acetaminophen (100 mg/kg) demonstrated an increased locomotor activity. Distance traveled was measured on the first drug pairing session. *Denotes a significant difference in distance traveled between drug and vehicle treated rats. $n = 6$ rats per group.

The combination of acetaminophen and hydrocodone is the most commonly prescribed opioid analgesic, therefore, in our experiments we combined varying doses of acetaminophen (50, 100 and 300 mg/kg) with hydrocodone (1.0 or 5.0 mg/kg) to examine whether the combinations would produce a greater rewarding effect as compared to hydrocodone given alone. Our results indicated that acetaminophen was able to enhance the rewarding effects of hydrocodone in a dose specific manner. In particular, acetaminophen (100 mg/kg) enhanced the rewarding property of a non-CPP producing dose of hydrocodone (1.0 mg/kg). This effect was not obtained when a lower or higher dose of acetaminophen (50 or 300 mg/kg) was combined with the same hydrocodone dose. These data suggest a unique dose requirement for acetaminophen to enhance hydrocodone reward; however, the precise mechanism underlying this effect is unclear.

Several mechanisms of action have been proposed for acetaminophen over the years. The most recent mechanism possessing data for the pain relieving properties of acetaminophen consist of the conversion of acetaminophen into *N*-(4-hydroxyphenyl)arachidonoylethanolamide (AM404) via two metabolic steps (Hogestatt et al., 2005). AM404 blocks the reuptake of the endocannabinoid anandamide (Beltramo et al., 1997), and is a TRPV1 receptor agonist (De Petrocellis et al., 2000; Zygmunt et al., 2000). Inhibition of anandamide transport and activation of TRPV1 receptors are suggested to modulate bulbospinal nociceptive pathways leading to antinociception (Mallet et al., 2008; 2010; Ruggieri et al., 2008); as well as increase dopamine release in the ventral striatum (Marinelli et al., 2005; Solinas et al., 2006), which may modulate reward processes. Based on this mechanism, one could expect acetaminophen to increase the rewarding effects of hydrocodone in a more consistent dose dependent manner; however, in our study acetaminophen did not increase the rewarding effects of hydrocodone in a dose dependent manner. Rather, only a single dose of acetaminophen (100 mg/kg) was effective. If the above-mentioned mechanism is contributing to our observed CPP effect, then such mechanism has a limited ability to modulate hydrocodone reward. This hypothesis is supported by our findings that the higher dose of acetaminophen (300 mg/kg) did not induce CPP when combined with lower doses of hydrocodone. Similarly, none of the doses of acetaminophen were able to potentiate CPP produced by a higher dose of hydrocodone (5.0 mg/kg). Lastly, 100 mg/kg of acetaminophen is considered a low analgesic and antihyperalgesic dose in rodent models of acute, inflammatory and neuropathic pain (Bonnefont et al., 2003; Hama and Sagen, 2010; Nagakura et al., 2003; Sandrini et al., 1999). Thus, the ability of acetaminophen to modulate the rewarding effects of hydrocodone may have limited physiological relevance.

The present study also measured the locomotor activating effects of hydrocodone, acetaminophen and their combination during the first drug conditioning session in the CPP paradigm. The highest dose of hydrocodone increased locomotor activity, indicative of acute locomotor activity. However, acetaminophen did not enhance hydrocodone-induced locomotor activity.

The increase in the non-medical use of prescription opioid analgesics has led to investigations in search of contributing factors that may explain the phenomenon. Although acetaminophen is formulated in the most commonly abused prescription opioid analgesics, it does not seem to have a noteworthy contribution in the rewarding effects of prescription opioid analgesics containing hydrocodone.

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