Enhancement of transdermal fentanyl and buprenorphine antinociception by transdermal Δ^9-tetrahydrocannabinol

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Abstract

Previous studies have demonstrated that Δ^9-tetrahydrocannabinol (THC) enhances the antinociceptive potency of many opioids administered by a variety of different routes of administration. We hypothesized that THC would enhance fentanyl or buprenorphine analgesia via the transdermal route of administration. THC was first demonstrated to enhance opioid antinociception when both drugs were administered parenterally in a hairless guinea pig model using the pin prick test. A low dose of THC (50 mg/kg, i.p.) produced no antinociception. However, THC enhanced the potency of s.c. fentanyl by 6.7-fold, and s.c. buprenorphine in a non-parallel fashion. For the transdermal studies, THC, fentanyl or buprenorphine was applied by pipette to the skin of the dorsum between the fore- and hind-flanks and covered with individual Tegederm™ patches. THC (400 mg/kg) produced no antinociception. However, THC enhanced fentanyl's potency by 3.7-fold at 2-h, and 5.8-fold at 4-h. Buprenophine's potency was increased 8.2-fold at 2-h and 7.2-fold at 4-h when co-administered with THC. These results indicate that the enhancement of transdermal opioids by THC could lead to the design of an effective combination analgesic patch.
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1. Introduction

The opioid fentanyl in the form of the Duragesic® patch (Janssen Pharmaceuticals) is prescribed by physicians to provide continuous pain relief, and often allows patients to be removed from intravenous pain medication. The patch releases fentanyl that penetrates the skin due to its lipophilicity, and is subsequently absorbed into systemic circulation. The resulting increase in plasma fentanyl concentrations is sufficient to provide sustained analgesia for up to 3 days. Fentanyl has been found to be 75–100 times more potent than morphine in the clinical setting (Donner and Zenz, 1995). Cancer and other chronic pain patients report higher satisfaction from fentanyl patches than from sustained-release oral morphine (Payne et al., 1998; Allan et al., 2001). Advantages associated with transdermal drug delivery include avoidance of first-pass metabolism, variable absorption, improved patient compliance and fewer side effects. However, several issues have arisen that limit the widespread use of the patches, including difficulty in titration of dose, potential for overdose, and the abuse liability of used patches.

In addition, many chronic cancer pain patients using the fentanyl patch are concurrently on Marinol®, an oral formulation of Δ^9-tetrahydrocannabinol (THC), for the treatment of nausea and vomiting associated with cancer chemotherapy. Marinol® is currently a Schedule III drug according to the U.S. Drug Enforcement Agency guidelines, but is not approved by the U.S. Food and Drug Administration for the treatment of pain. Laboratory investigations in animals have demonstrated that THC at high doses produces antinociception. However, very low doses of THC greatly enhance the antinociceptive effects of many classes of opiates such as morphine, fentanyl and methadone via oral and parenteral routes of administration (Smith et al., 1998; Cichewicz et al., 1999; Cichewicz and McCarthy, 2003), but transdermal delivery of these combinations has not yet been investigated.

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Buprenorphine, a derivative of the thebaine alkaloid resembling morphine, is a very potent opioid with properties of a partial agonist, in that its maximal effect is lower than that of morphine and it has antagonistic properties at delta- and kappa-opioid receptors (Heel et al., 1979; Leander, 1988). With high affinity for the mu-opioid receptor and a long duration of action, buprenorphine achieves its maximal antinociceptive effect slowly and with gradual receptor dissociation (Boas and Villiger, 1985). In contrast to morphine, buprenorphine is a weak reinforcer in humans, leading to a very low potential for physical dependence and moderate to slight withdrawal effects; this led to its use as an alternative to methadone in opioid addiction therapy (Mello et al., 1993; Ling et al., 1998; Litten and Allen, 1999). Its high lipophilicity results in a rapid penetration of the blood–brain barrier and high potency; clinically, buprenorphine can be about 25-fold more potent than morphine (Heel et al., 1979). Thus, its use as an analgesic is highly important, especially in morphine-sensitive subjects.

Due to its high lipophilicity, buprenorphine is an ideal candidate for a transdermal formulation as it can easily penetrate the skin (Evans and Easthope, 2003). A slow-release buprenorphine patch (Transtec™) is currently available for moderate to severe pain. However, buprenorphine is not the drug of choice for chronic cancer pain due to a proposed "ceiling" effect in which the analgesic efficacy plateaus at a submaximal level (De Castro et al., 1991). Still, buprenorphine's use as a long-term analgesic seems promising, since tolerance rapidly develops to its respiratory depressant effects compared to morphine and other opioids.

Fentanyl patches have been tested in animal models for post-surgical pain with favorable results: the animals showed only mild pain and the blood levels of fentanyl mimicked human serum levels (Gilbert et al., 2003). The hairless guinea pig model has been used successfully for transdermal drug application including latex allergy, contact dermatitis and photodermatology (Miyauchi and Horio, 1992; Hayes et al., 2000). The guinea pig provides an ideal skin surface without the need to shave the area of application, and is the best model similar to human skin. The goal of this study was to determine in hairless guinea pigs whether transdermally administered THC would enhance the antinociceptive potency of transdermal fentanyl and buprenorphine. Our results indicate that both THC and the opioids are effectively absorbed through the skin, and appear to interact to enhance opioid antinociception.

2. Methods

2.1. Animals

Female IAF hairless guinea pigs (Charles River Laboratories, Wilmington, MA) weighing 350–600 g were housed in individual cages in animal care quarters maintained at 22 ± 2 °C on a 12-h light–dark cycle. Animals were gonadally intact and estrous cycles were not considered in this study. Instead testing was conducted over many months, on a random basis throughout the month using different groups of guinea pigs that arrived at the facility. Testing on random days, of random doses of different drugs, was intended to blend any potential esterous cycle effects, since the intention of this study was not to examine the influence of esterous cycles on opioid sensitivity. Food and water were available ad libitum. All procedures were in accordance with regulations of the Institutional Animal Care and Use Committee of Virginia Commonwealth University, as well as the European Community guidelines for the use of experimental animals.

2.2. Pin prick test

Rats, guinea pigs, rabbits, dogs and other quadrupeds have a well-developed skin-flinch response to the nociceptive pin prick test (Morrow and Casey, 1983; Kramer et al., 1996; Hains et al., 2000; Khodorova and Strichartz, 2000). When the cutaneous skin of the dorsum is pricked with a 2 cm pin from a Buck neurological hammer (without penetrating the striatum corneum), a reflex movement of the skin is immediately observed. The Buck pin was developed for neurological assessment on humans, and the tip is sufficiently blunted to minimize the likelihood of penetrating intact skin. The skin-flinch reflex in guinea pigs (Blight et al., 1990) or other animals is not susceptible to fatigue from repeated stimuli. These movements are produced by contraction of subdermal muscles collectively called cutaneous trunk muscles (various names include cutaneous trunci, panniculus carnosus, cutaneous maximus) that line the dorsum and sides of the animal. The guinea pigs were held comfortably in the left hand and arm by the technician while the right hand was used to apply the pin. Nociception was indicated by a skin-flinch or by a nocifensive (i.e., startle or attempt to escape) response from the guinea pig. The 2 cm pin was randomly applied to various regions of the skin above and below and adjacent to the Tegederm™ patches on the upper and lower flanks. The area inside the patches was avoided to prevent disturbing the drug contained under the patch. Enough pressure was applied to elicit 10 baseline nociceptive responses before drug application, as described by Khodorova and Strichartz (2000). After drug administration, the test was repeated with 10 pin applications, and the percent inhibition of nocifensive responding was calculated by: $1 -$ [test responses/base responses] $\times 100$.

2.3. Parenteral administration of THC and opioids

Dose–response curves for fentanyl citrate and buprenorphine antinociception in the pin prick test were determined by administering various doses of fentanyl (30–100 µg/kg) and buprenorphine (1–5 mg/kg) s.c. in the skin on the back of the neck and testing 20–30 min later. The doses were injected on a 1 ml/kg volume so that the concentrations were equivalent to doses (e.g., 1–5 mg/kg=1–5 mg/ml). Time-course studies indicated that these were the peak times for s.c. opioid antinociception. In a separate experiment, 50 mg/kg THC i.p. was determined to have no activity in the pin prick test. Thus,
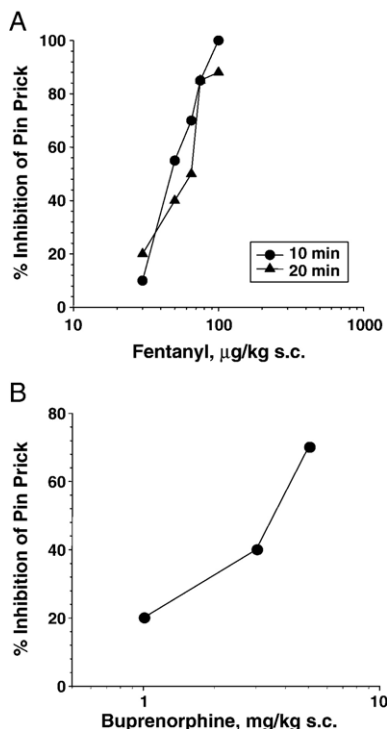
Fig. 1. (A) Fentanyl s.c. produces dose-dependent antinociception in the pin prick test. Hairless IAF guinea pigs were tested for baseline pin prick by applying pressure with a pin in 10 random spots on the dorsal surface (10 out of 10 trials caused a reflexive skin-flinch and nocifensive response). Fentanyl was administered s.c. in the skin of the back and the animals were tested 10 min (●) and 20 min (▲) later for pin prick. All data is presented as percent inhibition of pin prick. Each point represents 2–6 guinea pigs. (B) Buprenorphine s.c. produces dose-dependent antinociception in the pin prick test. Buprenorphine (●) was administered s.c. in the skin of the back and the animals were tested 30 min later for pin prick. All data is presented as percent inhibition of pin prick. Each point represents 2–6 guinea pigs.

for the enhancement studies, vehicle or THC (50 mg/kg) was administered i.p. prior to fentanyl or buprenorphine s.c, and the animals were tested 10-, 20- or 30-min later.

2.4. Transdermal administration of THC and opioids

Each animal was cleaned with soap and water followed by 70% ethanol to remove dead skin cells from the dorsal area. The study was designed to use a pipette to add a maximum of 100 μl of vehicle or opioid to the dorsal skin surface between the fore-and hind-flanks of a 0.6 kg guinea pig. Since dose–response curves were generated, the following range of doses was tested as well as the corresponding concentrations: fentanyl base (500 to 3000 μg/kg = 300 to 1800 μg/100 μl) and buprenorphine HCl (70 to 300 mg/kg = 42 to 180 mg/100 μl). THC or vehicle was added to the skin in a volume of 150 μl (240 mg/150 μl). A Tegederm™ patch (3.5 × 3.5 cm for opioids, 5.5 × 7.5 cm for THC, based on spread of the solutions) was applied to cover the area of drug administration and the animal was returned to its cage until testing at various time points. The 100 μl volume and 150 μl volume completely covered the patch, except in the outer margins that were adhered to the skin to prevent leakage of the drug

from under the patch. The patches were not assessed for drug content after completion of the study. When both drugs were applied simultaneously, each drug was covered by a separate patch, and oriented along the trunk of the body along the dorsum in a tandem configuration from fore- to hind-flank.

2.5. Statistical analysis

Effective dose-50 (ED_{50}) values and 95% confidence limits (C.L.) were calculated using least squares linear regression analysis as described by Bliss (1967). Dose–response curves were considered significantly different if the 95% C.L. did not overlap. Tests for parallelism were conducted by calculation of potency ratio values and 95% C.L. by the method of Colquhoun (1971). A potency ratio value of greater than 1, with a lower 95% C.L. greater than 1, was considered a significant difference in potency. For some treatment comparisons as indicated in the text, one-way analysis of variance (ANOVA) was conducted followed by post hoc analysis using the Tukey's test.
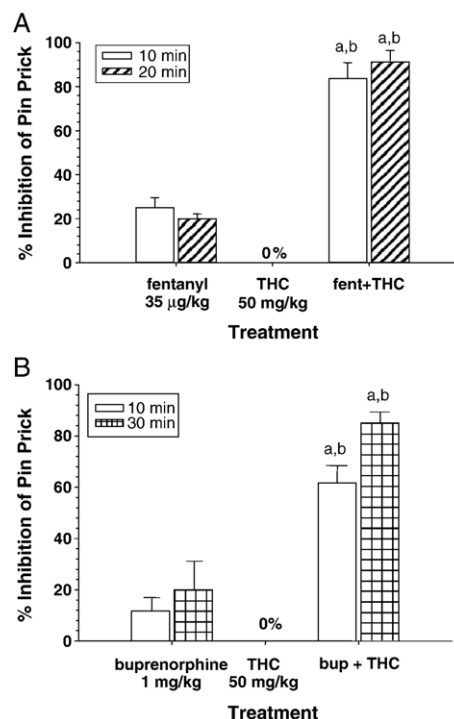

Fig. 2. (A) Low doses of THC and fentanyl produce synergistic antinociception at 10 and 20 min in the pin prick test. THC (50 mg/kg) was administered i.p. 20 min prior to fentanyl s.c. (35 μg/kg). The animals were tested 10 min (□) and 20 min (▨) later in the pin prick test. All data is presented as percent inhibition of pin prick. Each bar represents 6–8 guinea pigs. [a]Significantly different than opioid alone ($P < 0.01$); [b]significantly different than THC alone ($P < 0.01$), ANOVA and Tukey's test. (B) Low doses of THC and buprenorphine produce synergistic antinociception at 10 and 30 min in the pin prick test. THC (50 mg/kg) was administered i.p. immediately prior to buprenorphine s.c. (1 mg/kg). The animals were tested 10 min (□) and 30 min (▦) later for pin prick. Each bar represents 6–8 guinea pigs. [a]Significantly different than opioid alone ($P < 0.01$); [b]significantly different than THC alone ($P < 0.01$), ANOVA and Tukey's test.

2.6. Drugs

THC was obtained from the National Institute on Drug Abuse (NIDA) and was dissolved in 1 : 1 : 18 (emulphor, ethanol, saline) for i.p. administration and in 30% ethanol/70% dimethylsulfoxide (DMSO) for topical application. Fentanyl citrate (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile isotonic saline for s.c. administration. Fentanyl base converted from fentanyl citrate was suspended in 30% ethanol/ 70% DMSO for topical application. Buprenorphine hydrochloride (NIDA) was dissolved in sterile isotonic saline for s.c. administration, and was suspended in 30% ethanol/70% DMSO for transdermal application.

3. Results

3.1. Fentanyl and buprenorphine antinociception via s.c. administration

Dose–response curves were generated to determine the ED_{50} value of s.c. administered fentanyl and buprenorphine in the hairless guinea pig. As seen in Fig. 1A, fentanyl produced dose-dependent antinociception at both 10 and 20 min when administered s.c. The ED_{50} values of fentanyl were 49.1 µg/kg (95% C.L. 43.0 to 56.0) at 10 min, and 50.8 µg/kg (95% C.L.
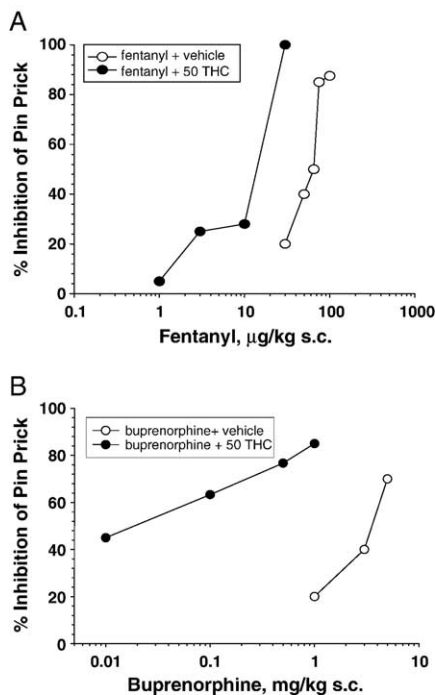
Fig. 3. (A) THC i.p. enhances the antinociceptive potency of fentanyl s.c. in the pin prick test. Vehicle (○) or THC (50 mg/kg; ●) was administered i.p. 20 min prior to injection of fentanyl s.c. The animals were tested 20 min following drug treatment for pin prick. (B) THC i.p. enhances the antinociceptive potency of buprenorphine s.c. in the pin prick test. Vehicle (○) or THC (50 mg/kg; ●) was administered i.p. immediately prior to injection of buprenorphine s.c. The animals were tested 30 min following drug treatment for pin prick. All data is presented as percent inhibition of pin prick. Each point represents 2–4 guinea pigs.
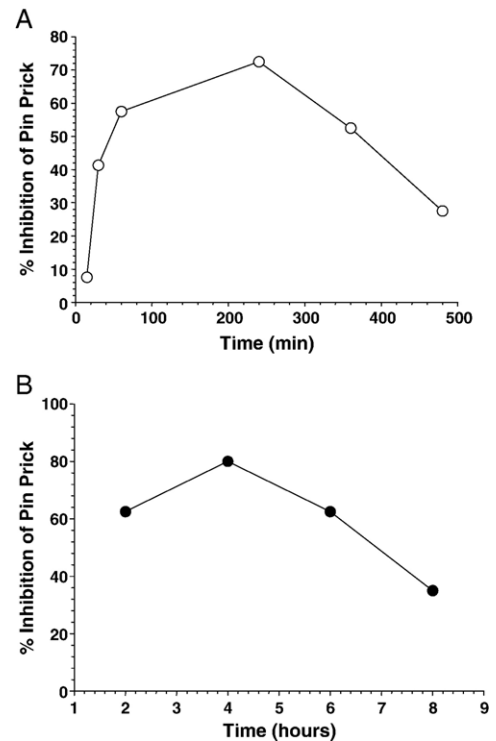
Fig. 4. (A) The antinociceptive effect of transdermal fentanyl persists for 8-h in the pin prick test. Fentanyl (1500 µg/kg; ○) was administered by pipette onto the skin on the surface of the back and covered with a Tegederm™ adhesive patch. The animals were tested at various time points following drug administration for pin prick. All data is presented as percent inhibition of pin prick. Each point represents 6–8 guinea pigs. (B) The antinociceptive effect of transdermal buprenorphine persists for 8-h in the pin prick test. Buprenorphine (30 mg/kg; ●) was administered by pipette onto the surface of the back and covered with a Tegederm™ adhesive patch. The animals were tested at various time points following drug administration for pin prick. Each point represents 4–8 guinea pigs.

41.0 to 63.0) at 20 min. Similarly, as seen in Fig. 1B, buprenorphine produced dose-dependent antinociception at 30 min, with an ED_{50} value of 3.0 mg/kg (95% C.L. 1.8 to 4.8). The antinociceptive effect of these drugs (reduction in nocifensive responding) was not believed to be due to generalized disruption of motor function, since this reflex was unaffected in guinea pigs sedated with benzodiazepines (midazolam), or anesthetized with pentobarbital (data not shown).

3.2. THC i.p. enhances s.c. opioid-induced antinociception

The hypothesis was tested that a low dose of THC (i.e., antinociception not elicited) would significantly enhance the antinociceptive effects of fentanyl and buprenorphine in the pin prick test. As seen in Fig. 2A, fentanyl (35 µg/kg, s.c.) produced a 25% inhibition or less of the pin prick response after 10 and 20 min, while THC (50 mg/kg, i.p.) produced no antinociception. However, co-administration of fentanyl and THC at their respective times resulted in a greater-than-additive effect on antinociception ($P < 0.01$). Similarly, buprenorphine (1 mg/kg s.c.) produced 20% inhibition or less of the pin prick response after 10 and 30 min (Fig. 2B). However, co-
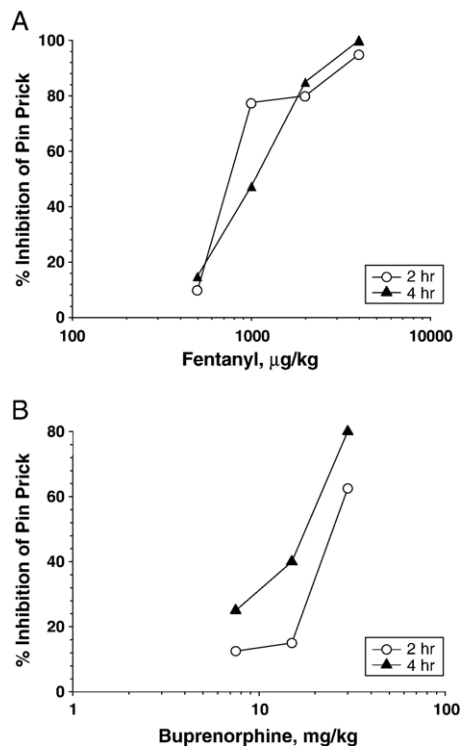
Fig. 5. (A) Transdermal fentanyl produces dose-dependent antinociception in the pin prick test. Fentanyl was administered by pipette onto the skin on the surface of the back and covered with a Tegederm™ adhesive patch. The animals were tested 2-h (○) and 4-h (▲) later for pin prick. All data is presented as percent inhibition of pin prick. Each point represents 4–6 guinea pigs. (B) Transdermal buprenorphine produces dose-dependent antinociception in the pin prick test. Buprenorphine was administered by pipette onto the skin on the surface of the back and covered with a Tegederm™ adhesive patch. The animals were tested 2-h (○) and 4-h (▲) later for pin prick. All data is presented as percent inhibition of pin prick. Each point represents 4 guinea pigs.

administration of buprenorphine and THC at their respective times resulted in a greater-than-additive effect on antinociception ($P < 0.01$).

THC also enhanced the antinociceptive potency of fentanyl and buprenorphine. The ED_{50} value of fentanyl s.c. was 50.8 μg/kg (41.0 to 63.0) when the pigs were tested 20 min later. A low dose of THC (50 mg/kg, i.p.) administered 20 min before fentanyl significantly decreased the ED_{50} value of fentanyl to 6.8 μg/kg (3.3 to 14.2), while the potency ratio was significant at 6.7 (1.8 to 17.0) (Fig. 3A). The ED_{50} value of buprenorphine s.c. was 2.97 mg/kg (1.84 to 4.81) when the pigs were tested 30 min later. In similar fashion, THC (50 mg/kg, i.p.) decreased the ED_{50} value to 0.02 mg/kg (0.01 to 0.05). However, it is not possible to statistically compare the change in potency produced by THC due to the non-parallel nature of the two dose–response curves (Fig. 3B).

3.3. Time-course of transdermal fentanyl and buprenorphine antinociception

Experiments were conducted to determine the time-course of the antinociceptive effects of transdermally absorbed fentanyl and buprenorphine. A 1500 μg/kg fentanyl dose in a 100 μl

bolus was added by pipette to the skin of the dorsum between the fore- and hind-flanks and covered by a Tegederm™ patch. Fentanyl absorption resulted in increasing antinociception during the first 4-h, which then began to decline over the 8-h test period (Fig. 4A). Similarly, buprenorphine (30 mg/kg) antinociception also peaked at 4-h, and declined over the 8-h test period. The 2- and 4-h time points were subsequently used for further experiments, based on the duration of action of fentanyl and buprenorphine.

3.4. Dose-dependent opioid antinociception via transdermal administration

Dose–response curves were generated to determine the ED_{50} values of fentanyl and buprenorphine by the transdermal route
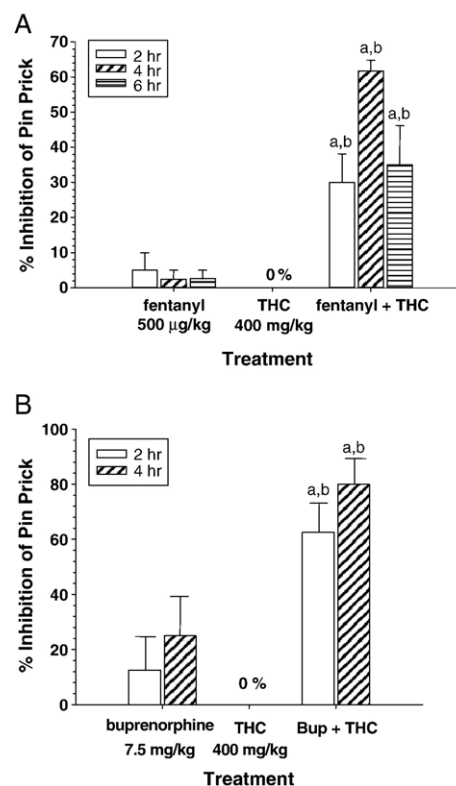

Fig. 6. (A) Low doses of transdermal fentanyl THC produce synergistic antinociception in the pin prick test. Fentanyl (500 μg/kg) was administered by pipette onto the skin in the center of the back and covered with a Tegederm™ adhesive patch. THC (400 mg/kg) was administered by pipette onto an adjacent section of the back and covered with a Tegederm™ adhesive patch. The animals were tested at 2-h □, 4-h ▨, and 6-h ▤ after drug administration for the pin prick test. All data is presented as percent inhibition of pin prick. Each bar represents 4–6 guinea pigs. [a]Significantly different than opioid alone ($P < 0.05$); [b]significantly different than THC alone ($P < 0.05$), ANOVA and Tukey's test. (B) Low doses of transdermal buprenorphine THC produce synergistic antinociception in the pin prick test. Buprenorphine (7.5 mg/kg) was administered by pipette onto the skin in the center of the back and covered with a Tegederm™ adhesive patch. THC (400 mg/kg) was administered by pipette onto an adjacent section of the back and covered with a Tegederm™ adhesive patch. The animals were tested at 2-h □ and 4-h ▨ after drug administration for the pin prick test. Each bar represents 4–6 guinea pigs. [a]Significantly different than opioid alone ($P < 0.01$); [b]significantly different than THC alone ($P < 0.01$), ANOVA and Tukey's test.

of administration. For these studies, 100 µl of drug at various doses was added by pipette and covered by a Tegederm™ patch. As seen in Fig. 5A, transdermal fentanyl produced dose-dependent antinociception at both 2- and 4-h. The ED_{50} values were 928.6 µg/kg (95% C.L. 599.5 to 1438.3) at 2-h and 1067.0 µg/kg (95% C.L. 840.4 to 1356.1) at 4-h. Transdermal buprenorphine also produced dose-dependent antinociception at both 2- and 4-h, with ED_{50} values of 26.1 mg/kg (95% C.L. 17.1 to 39.9) and 15.6 mg/kg (95% C.L. 10.0 to 24.5), respectively (Fig. 5B).

3.5. Enhancement of transdermal opioids by transdermal THC

The hypothesis was tested that a low dose of THC (i.e., antinociception not elicited) would significantly enhance the antinociceptive effects of transdermal fentanyl and buprenorphine in the pin prick test. As seen in Fig. 6A, transdermal fentanyl (500 µg/kg) produced 5% inhibition or less of the pin prick response after 2-, 4- or 6-h, while transdermal THC (400 mg/kg) produced no antinociception. However,

Table 1
Enhancement of the antinociceptive effects of transdermally administered fentanyl and buprenorphine by transdermal THC in the pin prick test

Treatment	Opioid ED_{50} (95% C.L.)	Potency ratio PR (95% C.L.)
2 h vehicle + fentanyl	928.6 µg/kg (599.5 to 1438.3)	–
THC (400 mg/kg) + fentanyl	254.9 µg/kg (202.6 to 320.6) [a]	3.7 (2.0 to 4.8) [a]
4 h vehicle + fentanyl	1067.0 µg/kg (840.4 to 1356.1)	–
THC (400 mg/kg) + fentanyl	176.3 µg/kg (144.3 to 215.5) [a]	5.8 (3.8 to 7.4) [a]
2 h vehicle + buprenorphine	26.1 mg/kg (17.1 to 39.9)	–
THC (400 mg/kg) + buprenorphine	4.3 mg/kg (2.8 to 6.8) [a]	8.2 (4.3–17.2) [a]
4 h vehicle + buprenorphine	15.6 mg/kg (10.0 to 24.5)	–
THC (400 mg/kg) + buprenorphine	2.2 mg/kg (1.1 to 4.6) [a]	7.2 (2.3 to 23.6) [a]

Vehicle or THC was pipetted onto the dorsal skin and covered with a Tegederm™ adhesive patch. Simultaneously, fentanyl or buprenorphine was pipetted onto the dorsal skin and covered with a separate patch. The animals were tested 2- and 4-h after drug administration in the pin prick test.
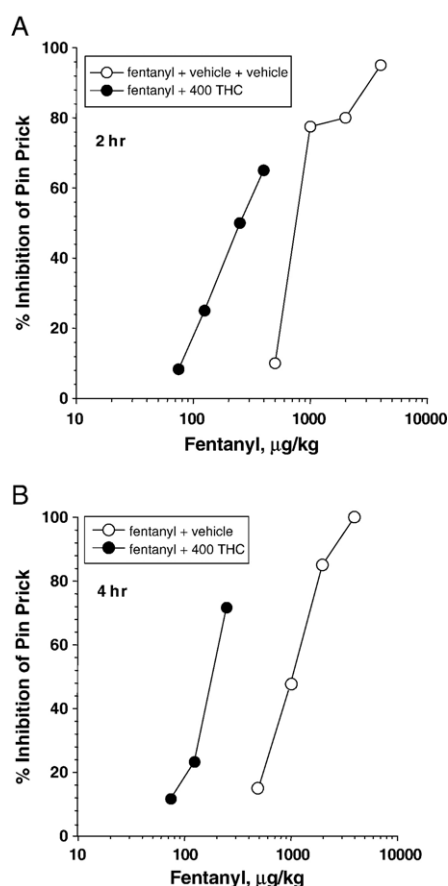[a] Significantly different from vehicle + opioid.

Fig. 7. Transdermal THC enhances the antinociceptive potency of transdermal fentanyl at 2-h (A) and 4-h (B). Fentanyl at various doses was administered by pipette onto the skin in the center of the back and covered with a Tegederm™ adhesive patch. Vehicle (○) or THC (400 mg/kg; ●) was administered by pipette onto an adjacent section of the back and covered with a Tegederm™ adhesive patch. The animals were tested at (A) 2-h and (B) 4-h after drug administration for pin prick. The data is presented as percent inhibition of pin prick. Each point represents 4–6 guinea pigs.

co-administration of fentanyl and THC under two separate patches resulted in a greater-than-additive effect on antinociception ($P < 0.05$). Similarly transdermal buprenorphine (7.5 mg/kg) produced 25% inhibition or less of the pin prick response after 2- and 4-h (Fig. 6B). However, co-administration of buprenorphine and THC under two separate patches resulted in a greater-than-additive effect on antinociception ($P < 0.01$).

Experiments were conducted to determine whether transdermal THC would enhance the potency of transdermal fentanyl and buprenorphine. As seen in Fig. 7A and B, THC (400 mg/kg) enhanced fentanyl's potency by 3.7-fold at 2-h and 5.8-fold at 4-h when both drugs were administered under separate patches (Table 1). In similar fashion, buprenorphine's potency was increased by 8.2-fold at 2-h and 7.2-fold at 4-h when THC (400 mg/kg) was co-administered under a separate patch (Fig. 8A, B, Table 1).

4. Discussion

The main goal of this study was to demonstrate that transdermal THC would enhance the antinociceptive potency of transdermal fentanyl and/or buprenorphine in an animal model. Previous studies from our laboratory revealed that parenterally administered THC and opioids (i.e., s.c, p.o.) can effectively increase the antinociceptive potency of many opioids, most notably morphine, hydromorphine, methadone and codeine (Smith et al., 1998; Cichewicz et al., 1999; Cichewicz and McCarthy, 2003). Less is known about the transdermal route of administration, although one recent study demonstrated synergy between the topically administered synthetic cannabinoid WIN55, 212-2 ((R)-(+)-[2,3-Dihydro-5-methyl-3-(4-
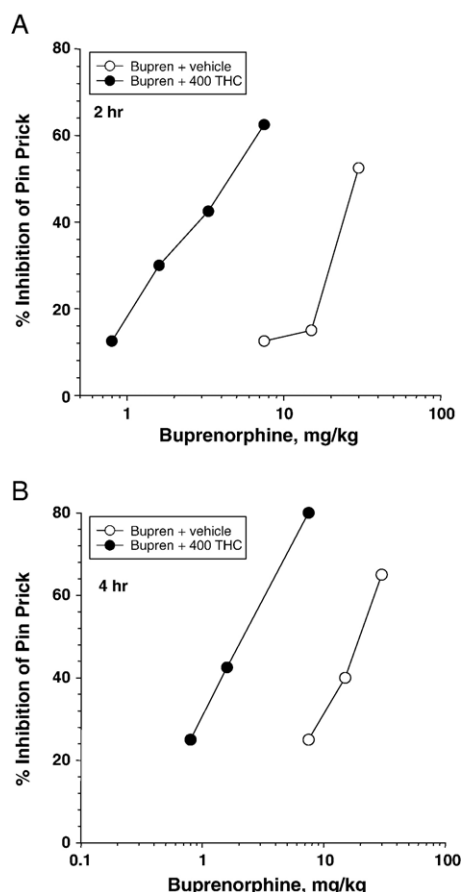
Fig. 8. Transdermal THC enhances the antinociceptive potency of transdermal buprenorphine at 2-h (A) and 4-h (B). Buprenorphine at various doses was administered by pipette onto the skin in the center of the back and covered with a Tegederm™ adhesive patch. Vehicle (○) or THC (400 mg/kg; ●) was administered by pipette onto an adjacent section of the back and covered with a Tegederm™ adhesive patch. The animals were tested at (A) 2-h and (B) 4-h after drug administration for pin prick. The data is presented as percent inhibition of pin prick. Each point represents 2–4 guinea pigs.

morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]]-1-naphthalenylmethanone) and morphine (Yesilyurt et al., 2003). The widespread use of fentanyl and buprenorphine patches for pain management spurred an in-depth examination of the potential utility of transdermally administered THC to provide high levels of antinociception when combined with transdermal opioid.

In the hairless guinea pig model, transdermal THC increased the potency of transdermal fentanyl by 3.7-fold after only 2-h. After 4-h, fentanyl's potency was increased by almost 6-fold. In humans, the fentanyl patch requires 8- to 12-h to reach steady state in the body, and bolus doses of supplemental opioid analgesic are often required to control pain (Gourlay et al., 1989). The results seen in the guinea pig indicate that THC enhanced the earliest antinociceptive effects of fentanyl. In humans, THC might also accelerate the development of fentanyl analgesia by its synergistic interaction with fentanyl. Thus, patients wearing a fentanyl-THC patch might not need supplemental opioid before plasma fentanyl levels achieve steady state.

Our studies clearly show that THC also enhanced the antinociceptive effects of buprenorphine. THC i.p. greatly increased the potency of buprenorphine but the curve was shifted to the left in a non-parallel fashion. Yet, antinociception was clearly enhanced, although the receptor mechanisms of this enhancement remain to be revealed. This enhancement is consistent with the accentuated analgesia seen with codeine, hydromorphine and methadone (Smith et al., 1998; Cichewicz et al., 1999; Cichewicz and McCarthy, 2003). Patients might benefit from high levels of buprenorphine analgesia after consuming THC by oral or other routes of administration. Buprenorphine is highly lipophilic and can permeate the skin quickly and effectively (Evans and Easthope, 2003). However, the chemical buprenorphine base was ineffective in the transdermal studies because of its tendency to crystallize. This observation has been made in the past (Stinchcomb et al., 1995). Thus, a switch to the hydrochloride salt provided the correct formulation for skin penetration. Finally, once transdermal absorption occurred, our results indicate that buprenorphine and THC interacted to enhance the antinociceptive potency of buprenorphine.

It could be argued that the 30% ethanol/70% DMSO vehicle was not very efficient at delivering the drugs transdermally, therefore requiring high concentrations of THC, fentanyl and buprenorphine in the patch to elicit antinociception. Transdermal patches typically contain high concentrations of drug so that a sufficient amount of drug is delivered across the skin to provide the same plasma concentrations that would be achieved with parenteral administration. For example, the 25 μg/h Duragesic® patch contains 2.5 mg of fentanyl base, which is slowly absorbed over several days. Immediate absorption of 2.5 mg from the Duragesic® patch in a 70 kg individual would result in a dose of 0.036 mg/kg, which is 18-times higher than the 0.002 mg/kg dose required to provide postoperative pain relief, or light anesthesia for minor surgical procedures. Similarly, the ED_{50} values required to elicit antinociception by topically applied fentanyl in the guinea pigs (i.e., 926 at 2-h and 1067 at 4-h μg/kg) were 18.2- and 21.0-fold times higher than the dose required to elicit antinociception to s.c. fentanyl (i.e., 50.8 μg/kg). These results indicate that the 30% ethanol/70% DMSO vehicle delivered the fentanyl topically with the same efficiency as the Duragesic® patch. Incorporation of bio-compatible excipients such as Transcutol™, glycofurol or miglyol 810 could significantly increase the transdermal delivery of fentanyl compared to the current Duragesic® patch, thereby decreasing the amount of fentanyl required in the patch. Furthermore, by exploiting the synergistic interaction between THC and opioids, smaller doses of fentanyl and THC would be needed to provide analgesia, thereby minimizing the potential diversion of a "Fentanyl-THC" patch.

These dose–response studies indicate that the cannabinoid and opioid systems interact in the production of antinociception. This concept has already been extensively demonstrated using various routes of drug administration (for review, see Cichewicz, 2004). The results presented here represent the first evidence that the combination of THC and fentanyl or

buprenorphine may be effective in a patch formulation. Previous work indicates that both opioid and cannabinoid receptors in the brain and spinal cord which activate similar signaling pathways via G protein interactions, are involved in the synergistic antinociception produced by THC and morphine (Cichewicz et al., 1999). Since THC is known to induce the release of dynorphin and enkephalin that act at kappa- and delta-opioid receptors in the spinal cord (Mason et al., 1999; Welch and Eads, 1999; Valverde et al., 2001), these peptides may interact synergistically with fentanyl and buprenorphine at mu-opioid receptors to enhance antinociception.

The benefits of transdermal drug application are numerous: providing an alternate route of administration for patients unable to swallow pills or suffering from emesis; utilizing a non-invasive, high compliance technique which can be accomplished outside the clinic; avoiding first-pass metabolism and yielding a pharmacokinetic profile comparable to that of i.v. administration; and maintaining steady-state plasma concentrations for prolonged periods of time (Caplan and Southam, 1990; Sittl et al., 2003). Finally, the ability of THC to enhance the potency of fentanyl and buprenorphine suggests that less opioid would need to be infused from any developed transdermal delivery system. This could diminish the likelihood of opioid side effects such as respiratory depression and chronic constipation, thereby increasing safety and long-term patient compliance.

Acknowledgements

References

Allan, L., Hays, H., Jensen, N.-H., Le Polain de Waroux, B., Bolt, M., Donald, R., Kalso, E., 2001. Randomised crossover trial of transdermal fentanyl and sustained release oral morphine for treating chronic non-cancer pain. Brit. Med. J. 322, 1–7.

Blight, A.R., McGinnis, M.E., Borgens, R.B., 1990. Cutaneus trunci muscle reflex of the guinea pig. J. Comp. Neurol. 296, 614–633.

Bliss, C.I., 1967. Statistics in Biology. McGraw-Hill, New York, NY, p. 439.

Boas, R.A., Villiger, J.W., 1985. Clinical actions of fentanyl and buprenorphine. The significance of receptor binding. Br. J. Anaesth. 57, 192–196.

Caplan, R.A., Southam, M., 1990. Transdermal drug delivery and its application to pain control. In: Benedetti, C. (Ed.), Advances in Pain Research and Therapy. Raven Press, New York, NY, pp. 233–240.

Cichewicz, D.L., 2004. Synergistic interactions between cannabinoid and opioid analgesics. Life Sci. 74, 1317–1324.

Cichewicz, D.L., McCarthy, E.A., 2003. Antinociceptive synergy between delta (9)-tetrahydro-cannabinol and opioids after oral administration. J. Pharmacol. Exp. Ther. 304, 1010–1015.

Cichewicz, D.L., Martin, Z.L., Smith, F.L., Welch, S.P., 1999. Enhancement of mu opioid antinociception by oral delta9-tetrahydrocannabinol: dose–response analysis and receptor identification. J. Pharmacol. Exp. Ther. 289, 859–867.

Colquhoun, D., 1971. Lectures on Biostatistics: An Introduction to Statistics with Applications in Biology and Medicine. Clarendon Press, Oxford, U.K., pp. 327–333.

De Castro, J., Meynadier, J., Zanz, M., 1991. Drugs. In: de Castro, J., Meynadier, J., Zanz, M. (Eds.), Regional Opioid Analgesia. Klumer Academic Publishers, Dordrecht, pp. 131–209.

Donner, B., Zenz, M., 1995. Transdermal fentanyl: a new step on the therapeutic ladder. Anticancer Drugs 6, 39–43.

Evans, H.C., Easthope, S.E., 2003. Transdermal buprenorphine. Drugs 63, 1999–2012.

Gilbert, D.B., Motzel, S.L., Das, S.R., 2003. Postoperative pain management using fentanyl patches in dogs. Contemp. Top. Lab. Anim. Sci. 42, 21–26.

Gourlay, G.K., Kowalski, S.R., Plummer, J.L., Cherry, D.A., Gaukroger, P., Cousins, M.J., 1989. The transdermal administration of fentanyl in the treatment of postoperative pain: pharmacokinetics and pharmacodynamic effects. Pain 37, 193–202.

Hains, B.C., Chastain, K.M., Everhart, A.W., McAdoo, D.J., Hulsebosch, C.E., 2000. Transplants of adrenal medullary chromaffin cells reduce forelimb and hindlimb allodynia in a rodent model of chronic central pain after spinal cord hemisection injury. Exp. Neural. 164, 426–437.

Hayes, B.B., Afshari, A., Millecchia, L., Willard, P.A., Povoski, S.P., Meade, B. J., 2000. Evaluation of percutaneous penetration of natural rubber latex proteins. Toxicol. Sci. 56, 262–270.

Heel, R.C., Brogden, R.N., Speight, T.M., Avery, G.S., 1979. Buprenorphine: a review of its pharmacological properties and therapeutic efficacy. Drugs 17, 81–110.

Khodorova, A.B., Strichartz, G.R., 2000. The addition of dilute epinephrine produces equieffectiveness of bupivacaine enantiomers for cutaneous analgesia in the rat. Anesth. Analg. 91, 410–416.

Kramer, S., Nolte, I., Jochle, W., 1996. Clinical comparison of medetomidine with xylazine/l-methadone in dogs. Vet. Rec. 138, 128–133.

Leander, J.D., 1988. Buprenorphine is a potent/cappa-opioid receptor antagonist in pigeons and mice. Eur. J. Pharmacol. 151, 457–461.

Ling, W., Charuvastra, C., Collins, J.F., Batki, S., Brown Jr., L.S., Kintaudi, P., Wesson, D.R., McNicholas, L., Tusel, D.J., Malkemeker, U., Renner Jr., J. A., Santos, E., Casadonte, P., Fye, C., Stine, S., Wang, R.I., Segal, D., 1998. Buprenorphine maintenance treatment of opiate dependence: a multicenter, randomized clinical trial. Addiction 93, 475–486.

Litten, R.Z., Allen, J.P., 1999. Medications for alcohol, illicit drug, and tobacco dependence. An update of research findings. J. Subst. Abuse Treat. 16, 105–112.

Mason Jr., D.J., Lowe, J., Welch, S.P., 1999. Cannabinoid modulation of dynorphin A: correlation to cannabinoid-induced antinociception. Eur. J. Pharmacol. 378, 237–248.

Mello, N.K., Mendelson, J.H., Lukas, S.E., Gastfriend, D.R., Teoh, S.K., Holman, B.L., 1993. Buprenorphine treatment of opiate and cocaine abuse: clinical and preclinical studies. Harv. Rev. Psychiatry. 1, 168–183.

Miyauchi, H., Horio, T., 1992. A new animal model for contact dermatitis: the hairless guinea pig. J. Dermatol. 19, 140–145.

Morrow, T.J., Casey, K.L., 1983. Suppression of bulboreticular unit responses to noxious stimuli by analgesic mesencephalic stimulation. Somatosens. Res. 1, 151–168.

Payne, R., Mathias, S.D., Pasta, D.J., Wanke, L.A., Williams, R., Mahmoud, R., 1998. Quality of life and cancer pain: satisfaction and side effects with transdermal fentanyl versus oral morphine. J. Clin. Oncol. 16, 1588–1593.

Sittl, R., Griessinger, N., Likar, R., 2003. Analgesic efficacy and tolerability of transdermal buprenorphine in patients with inadequately controlled chronic pain related to cancer and other disorders: a multicenter, randomized, double-blind, placebo-controlled trial. Clin. Ther. 25, 150–168.

Smith, F.L., Cichewicz, D., Martin, Z.L., Welch, S.P., 1998. The enhancement of morphine antinociception in mice by delta9-tetrahydrocannabinol. Pharmacol. Biochem. Behav. 60, 559–566.

Stinchcomb, A.L., Dua, R., Paliwal, A., Woodard, R.W., Flynn, G.L., 1995. A solubility and related physicochemical property comparison of buprenorphine and its 3-alkyl esters. Pharm. Res. 12, 1526–1529.

Valverde, O., Noble, F., Beslot, F., Dauge, V., Foumie-Zaluski, M.C., Roques, B.P., 2001. Delta9-tetrahydrocannabinol releases and facilitates the effects of endogenous enkephalins: reduction in morphine withdrawal syndrome without change in rewarding effect. Eur. J. Neurosci. 13, 1816–1824.

Welch, S.P., Eads, M., 1999. Synergistic interactions of endogenous opioids and cannabinoid systems. Brain Res. 848, 183–190.

Yesilyurt, O., Dogrul, A., Gul, H., Seyrek, M., Kusmez, O., Ozkan, Y., Yildiz, O., 2003. Topical cannabinoid enhances topical morphine antinociception. Pain 105, 303–308.