Antiallodynic Effects of Loperamide and Fentanyl against Topical Capsaicin-Induced Alloodynia in Unanesthetized Primates

Eduardo R. Butelman, Todd J. Harris, and Mary Jeanne Kreek

The Rockefeller University, New York, New York

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ABSTRACT

Capsaicin produces thermal alldynia in animals and humans by acting as an agonist at vanilloid receptor subtype 1 [VR1; also known as transient receptor potential vanilloid type 1 (TRPV1)]. VR1 receptors are widely distributed in the periphery (e.g., on primary afferent neurons). These studies examined the ability of loperamide (0.1–1 mg/kg s.c.; a μ-opioid agonist that is peripherally selective after systemic administration), in preventing and reversing thermal alldynia caused by topical capsaicin (0.004 M) in rhesus monkeys, within a tail withdrawal assay (n = 4; 38°C and 42°C; normally non-noxious thermal stimuli). The effects of loperamide were compared with those of the centrally penetrating μ-agonist, fentanyl (0.0032–0.032 mg/kg s.c.). We also characterized the allodynic effects of the endogenous VR1 agonist (“endovanilloid”), N-oleoyldopamine (OLDA; 0.0013–0.004 M). In this model, loperamide and fentanyl produced dose-dependent prevention of capsaicin-induced alldynia, whereas only fentanyl produced robust reversal of ongoing alldynia. Antagonism experiments with naltrexone (0.1 mg/kg s.c.) or its analog, methylnaltrexone (0.32 mg/kg s.c.), which does not readily cross the blood-brain barrier, suggest that the antiallodynic effects of loperamide and fentanyl were predominantly mediated by peripherally and centrally located μ-receptors, respectively. Loperamide and fentanyl (1 mg/kg and 0.032 mg/kg, respectively) also prevented OLDA (0.004 M)-induced alldynia. Up to the largest dose studied, loperamide was devoid of thermal antinociceptive effects at 48°C (a noxious thermal stimulus, in the absence of capsaicin). By contrast, fentanyl (0.01–0.032 mg/kg) caused dose-dependent antinociception in this sensitive thermal antinociceptive assay (a presumed centrally mediated effect). These studies show that loperamide, acting as a peripherally selective μ-agonist after systemic administration, can prevent capsaicin-induced thermal alldynia in primates in vivo, in the absence of thermal antinociceptive effects.

Capsaicin is the main pungent component of “hot” chili peppers; this compound can produce thermal alldynia in experimental animals and humans, by acting as an agonist at vanilloid receptors subtype 1 (VR1; also known as TRPV1 receptors) (Caterina and Julius, 2001). VR1 receptors are widely distributed in the periphery and in the central nervous system (Mezey et al., 2000). With respect to cutaneous tissues, as relevant to these studies, VR1 receptors are located in several structures, including primary afferent neurons and keratinocytes (Caterina and Julius, 2001; Southall et al., 2003). Vanilloid VR1 receptors are sensitized and/or up-regulated during conditions associated with tissue damage, including noxious heat, low pH, inflammation, or neuropathic insults (Caterina and Julius, 2001; Hudson et al., 2001; Ji et al., 2002). As such, capsaicin-induced alldynia and its modulation are prominent models in the study of pain mechanisms in vivo and their pharmacological blockade.

Studies in rodents have indicated that μ-opioid receptors are located in the periphery (e.g., on primary afferents), where they may modulate ascending painful stimuli (Andrew et al., 1994). It has also been reported that μ-opioid receptor populations may increase in density in local neuronal sites following inflammatory or neuropathic insults, in rodents (Truong et al., 2003; Zollner et al., 2003). Thus, “peripherally selective” μ-opioid agonists or locally administered μ-opioid agonists may be investigated for their potential to block painful conditions, in the relative absence of the undesirable effects of systemically administered, centrally penetrating μ-agonists (e.g., respiratory depression, pruritus, cognitive effects) (DeHaven-Hudkins et al., 1999; Twillman et al., 1999; O’Mahony et al., 2001; Ko et al., 2004).

ABBREVIATIONS: VR1, vanilloid receptor subtype 1; OLDA, N-oleoyldopamine; ANOVA, analysis of variance.
In these studies, we therefore compared loperamide (which acts as a peripherally selective µ-agonist after parenteral administration) with fentanyl, a centrally penetrating µ-agonist, in a recently developed model of topical capsaicin-induced thermal allodynia in unanesthetized rhesus monkeys (Butelman et al., 2003). Loperamide’s peripheral selectivity after parenteral administration is likely due to its property as a substrate for a P-glycoprotein (multidrug resistance) transporter, located in the blood-brain barrier (Schinkel et al., 1996; Wandel et al., 2002). Therefore, although loperamide is a lipophilic molecule of moderate molecular weight (mol. wt. = 513.5), it is removed from potential accumulation at central sites by this transporter. Both loperamide and fentanyl have binding selectivity for µ- over κ- or δ-receptors in vitro, and both are also highly efficacious µ-agonists (Toll et al., 1997; DeHaven-Hudkins et al., 1999).

For example, loperamide’s binding affinity at cloned human µ-receptors exhibits a \( K_i \) of 3.3 nM (DeHaven-Hudkins et al., 1999). Loperamide also has lower affinity at calcium channels labeled by \((-)\[^3^H\]desmethoxyverapamil in vitro, with \( IC_{50} \) values in the 100 to 200 nM range; a loperamide-induced blockade of calcium-channel currents has also been observed in vitro (Reynolds et al., 1986; Church et al., 1994).

Prior allodynia/hyperalgesia studies in unanesthetized primates have focused on injected, rather than topical capsaicin or other agents (Ko et al., 1998b; Brandt et al., 2001). Interestingly, injected and topical capsaicin may produce somewhat different effects on sensory neurons in vivo (LaMotte et al., 1992). For example, a greater degree of within-session desensitization of primary afferent function was reported after injected versus topical capsaicin in humans (LaMotte et al., 1992). Both injected and topical modes of capsaicin administration have been used as experimental pain stimuli in humans (LaMotte et al., 1992; Anderson et al., 2002). In this study, we examined the relative ability of loperamide and fentanyl to modulate thermal allodynia. We also compared the effects of capsaicin in this model with those of the recently discovered "endovanilloid" (endogenous VR1 agonist) OLDA (Chu et al., 2003). These are the first studies, to our knowledge, that directly compare the antiallodynic effects of systemically administered peripherally selective and centrally penetrating nonpeptidic µ-agonists in primates. These studies also provide, to our knowledge, the first evaluation in primates of the allodynic effects of the recently discovered endovanilloid, OLDA.

## Materials and Methods

### Subjects

Adult, gonadally intact rhesus monkey females (Macaca mulatta, age range 8–11 years, approximately) were used as subjects. They were singly housed in a room maintained at 20–22°C with controlled humidity, and a 12:12-h light/dark cycle (lights on at 7:00 AM). Experiments took place between the hours of 10:00 AM and 2:00 PM. Monkeys were fed approximately 11 jumbo primate chow biscuits (PMI Feeds, Brentwood, MO) daily, supplemented by fruit and multivitamins. Water was freely available in home cages, via an automatic waterspout. Unless otherwise stated, experiments were carried out with an \( n = 4 \). Before these studies, monkeys had been exposed several times to the experimental situation and were previously chair-trained with the standard "pole and collar" system. Monkeys had never received chronic administration of opioid compounds as part of their prior history.

### Procedures

#### Topical Capsaicin-Induced Thermal Allodynia

The present assay is a recent modification of the warm-water tail withdrawal assay, adapted to study topical capsaicin-induced allodynia, based on previous models in humans and primates, butelman et al., 1999). Their tails are shaved with standard clippers. Tail withdrawal latencies were timed manually in 0.1-s increments, up to a maximum (cutoff) latency of 20 s. Baseline latencies are determined in 38°C and 42°C water stimuli (thermal stimuli are used within ±0.3°C of the specified temperature). If a monkey did not remove its tail from the water by 20 s, the experimenter removed the water, and a 20-s value was assigned (38°C and 42°C thermal stimuli are normally non-noxious in human or nonhuman primates) (Culp et al., 1989). After baseline determination, the tail is gently dried and then degreased with an isopropyl alcohol pad. The topical capsaicin patch is applied (as described below) for 15 min. At the end of the topical capsaicin exposure, the patch is removed and testing in the above thermal stimuli occurs at standard intervals (5, 15, 30, 60, and 90 min after capsaicin removal). At each time point, the two thermal stimuli are tested in a nonsystematic order, with tests in the two stimuli separated from each other by approximately 2 min.

#### Topical Capsaicin/Endovanilloid Administration

A 1-cm² patch of two-ply gauze (Johnson & Johnson, Arlington, TX) is attached on waterproof adhesive backing (23-mm diameter; Active Strips; 3M Health Care, St. Paul, MN) (Culp et al., 1989; Kupers et al., 1997). This backing is in turn attached onto elastic adhesive tape (5 cm wide; Elastikon; Johnson & Johnson). Capsaicin (typically 0.004 M) is dissolved in a vehicle composed of 70% ethanol and 30% sterile water by volume, approximately 30 min before use. Capsaicin (0.3 ml of the above solution) is slowly injected onto the gauze patch, saturating the patch, and minimizing overflow. Within 30 s of the capsaicin solution being added, the patch is fastened onto the tail skin by means of the surrounding tape (e.g., 2–6 cm from the distal end). The patch is removed after 15 min of exposure, and this is followed by tail withdrawal testing as described above. Allodynia is detected as a decrease in tail withdrawal latency from normally non-noxious thermal stimuli (i.e., 35°C and 42°C). Consecutive sessions with topical capsaicin in the same subject were typically separated by at least 7 days. In selected experiments, the recently discovered endovanilloid, OLDA (0.0013–0.004 M) (Chu et al., 2003), was used topically instead of capsaicin (all other conditions are as described above; the 0.004 M OLDA concentration is near the solubility limit under these conditions).

#### Antiallodynia Assay

In antiallodynia prevention studies, a single dose of a compound (loperamide or fentanyl) was administered subcutaneously to prevent allodynia caused by topical capsaicin or OLDA (0.004 M), administered as described above. In antiallodynia reversal studies, a single dose of a compound (loperamide or fentanyl) was administered subcutaneously after capsaicin-induced allodynia was ongoing (i.e., approximately 15 min after the removal of topical capsaicin). This post-treatment was followed by testing at the remaining time points (30, 60, and 90 min), as above.

#### Assay of Thermal Antinociception

In separate studies, the thermal antinociceptive effects of loperamide (0.32–1 mg/kg) or fentanyl (0.01–0.032 mg/kg) were studied for comparison. The main purpose of these probe studies was to determine whether antiallodynic (above) and antinociceptive effects of these compounds are encountered at the same doses and times. The assay is identical to that above, except that monkeys are tested in the absence of capsaicin treatment, and are tested in 42°C (non-noxious) and 48°C (noxious) thermal stimuli. In pilot studies, 48°C was determined to be the least intense thermal stimulus that would elicit rapid tail withdrawal responses from all the present subjects (not shown). After baseline determination, subjects were injected with s.c. vehicle (0.1 mg/kg) or drug and then tested at the remaining time points (30, 60, and 90 min), as above.
ml/kg) or a single s.c. dose of loperamide or fentanyl in the scapular region. This was followed by measurement of tail withdrawal latencies at standard time points, up to 90 min after injection.

**Design**

**Allodynia Studies.** Studies were carried out in a single determination (n = 4), unless otherwise stated.

Capsaicin or OLDA-induced allodynia. The effects of topical vehicle and topical capsaicin (0.004 M) applied on the tail were studied in separate experiments. Topical capsaicin (0.004 M) was studied in two baseline determinations, separated from each other by approximately 2 months (with other intervening experiments occurring during this period, at approximately 1-week intervals). In one of these baseline determinations, topical capsaicin was studied alone, and in the other determination, capsaicin was studied after systemic s.c. vehicle pretreatment. The alldynic effects of OLDA (0.0013 and 0.004 M) were studied after topical administration on the tail, under identical conditions (Chu et al., 2003). The lower OLDA concentration (0.0013 M) was studied in one determination, and the higher OLDA concentration (0.004 M) was studied in two determinations, as described above.

**Effects of loperamide and fentanyl.** The effectiveness of loperamide (0.1–1 mg/kg s.c.) and fentanyl (0.01–0.032 mg/kg s.c.) in preventing capsaicin-induced allodynia was studied. Loperamide was administered 30 min before the removal of the topical capsaicin patch, whereas fentanyl was administered 15 min before the removal of the topical capsaicin patch. These dose ranges and times were based on prior available studies with parenterally administered loperamide and fentanyl in this species (Yanagita et al., 1979; Negus and Mello, 1999; Ko et al., 2002). In separate antagonism studies, the antiallodynic effect of the largest loperamide and fentanyl dose was studied 30 min after pretreatment with either naltrexone (0.1 mg/kg) or methylaltrexone (0.32 mg/kg), followed by testing as above. The selection of naltrexone and methylaltrexone doses was based on available prior studies on the effects of these antagonists in this species, and in humans (Ko et al., 1998a; Yuan et al., 2002; Butelman et al., 2004). The timing of these sessions is further illustrated in Table 1.

In separate studies, the effectiveness of the largest dose of loperamide and fentanyl (1 mg/kg and 0.032 mg/kg, respectively) in reversing capsaicin-induced allodynia was studied (compared with vehicle). In these studies, capsaicin (0.004 M) was administered as above, with tests at 5 and 15 min after removal of the capsaicin patch. Immediately after the 15-min test, the subjects were injected with loperamide or fentanyl, and this was followed by standard testing at the remaining time points (30, 60, and 90 min after capsaicin removal).

**Antinociception Studies.** The thermal antinociceptive effects of loperamide (0.32–1 mg/kg) and fentanyl (0.01–0.032 mg/kg) were studied against a 48°C determination in 38°C and 42°C water. The apparent onset of peak allodynia caused by capsaicin in this species over the present dose range (Negus and Mello, 1999).

**Data Analysis**

Tail withdrawal latency was the dependent variable in these studies. This was obtained from 38°C and 42°C in the presence of capsaicin or OLDA (antiallodynia), or from 48°C in the absence of capsaicin (antinociception). Data are presented graphically as mean ± S.E.M. Data were analyzed in either one or two-way repeated measures ANOVAs, followed by Dunnett’s or Newman-Keuls tests (SPSS-Sigmastat and GraphPad Prism). The level of significance (α) was set at the 0.05 level throughout. In selected cases, antiallodynic or antinociceptive potency is defined as ED_{10}, values (i.e., the agonist dose that would result in a 10-s latency; similar to ED_{50}, given the present 20-s cutoff). Such ED_{10} values were calculated by linear regression of individual log dose points above and below the 10-s level of effect (95% confidence limits were calculated as ±S.E.M.) (Kenakin, 1993).

**Test Compounds**

Naltrexone HCl (kindly supplied by the National Institutes of Health-National Institute on Drug Abuse Drug Supply System, Baltimore, MD), naltrexone methobromide (methylaltrexone; kindly supplied by Dr. C.S. Yuan, Department of Anesthesiology, University of Chicago, Chicago, IL), and fentanyl citrate (Siga-Aldrich, St. Louis, MO) were dissolved in sterile water. Loperamide HCl (Siga-Aldrich) was dissolved in ethanol (10%/Tween 80 (10%)/sterile water (80%). Capsaicin (98% pure; Sigma-Aldrich) and N-oleyl dopamine (OLDA; Tocris Cookson Inc., Ellisville MO) were prepared in ethanol (70%/sterile water (30%) vehicle, approximately 30 min before topical use. The pH of all topically applied solutions was approximately 7. Doses of all compounds are in the forms described above.

**Results**

**Effects of Topically Administered Vehicle, Capsaicin, or OLDA.** Baseline tail withdrawal latencies in 38°C and 42°C water uniformly reached cutoff (20 s) in the present subjects. Tail administration of topical vehicle (70% ethanol) under the conditions described above did not affect tail withdrawal latencies over a standard 90-min test (Fig. 1). By contrast, topical administration of capsaicin on the tail (0.004 M; one determination alone and one determination after systemic s.c. vehicle pretreatment) caused robust allodynia (similar to previously reported determinations) (Butelman et al., 2003). In a pilot session, identical administration of topical capsaicin at a remote site (the calf area of the leg) did not result in any allodynia (not shown). These two determinations of capsaicin on the tail were made approximately 2 months apart (with other intervening capsaicin tests, at approximately 1-week intervals). In all graphs and analyses, the two above determinations with topical capsaicin (0.004 M) on the tail were averaged. One-way repeated measures ANOVAs for time after capsaicin (and pre-capsaicin baseline) yielded a significant effect of time in 38°C water [F(5,15) = 51.36] and 42°C [F(5,15) = 269.9].

The apparent onset of peak allodynia caused by capsaicin (0.004 M) was 15 to 30 min after the removal of the topical patch, as demonstrated by the presence of near-maximal allodynia in 42°C water, starting from the 15-min time point. In a single-probe determination, capsaicin (0.004 M)-induced allodynia was found to dissipate completely by 24 h after topical administration, at both 38°C and 42°C (not shown).

The endovanilloid OLDA (0.0013–0.004 M) caused concentration-dependent allodynia in 90-min tests. The larger
OLDA concentration was studied twice (one determination alone and one determination after systemic s.c. vehicle pretreatment); these two determinations were averaged for graphical and statistical purposes. A two-way (time × OLDA concentration) repeated measures ANOVA for 38°C water was significant for time \( F(4,12) = 9.55 \), treatment \( F(2,6) = 55.25 \), and their interaction \( F(8,24) = 8.76 \). A similar ANOVA for 42°C water also yielded significant effects of time \( F(4,12) = 3.35 \), treatment \( F(2,6) = 19.21 \), and their interaction \( F(8,24) = 3.46 \). However, a maximal degree of alldynia was observed with the largest OLDA concentration only at the more intense thermal stimulus (42°C; Fig. 1). A larger topical concentration of OLDA could not be studied under the present conditions, due to solubility limits. In a single-probe determination, OLDA (0.004 M)-induced alldynia was found to dissipate completely by 24 h after topical administration, at both 38°C and 42°C (not shown).

**Effects of Loperamide and Fentanyl in Preventing Capsaicin-Induced Alldynia.** Based on the present determinations (above) and prior concentration-effect studies (Butelman et al., 2003), the 0.004 M capsaicin topical treatment was selected as a standard alldynia-inducing stimulus.

Loperamide (0.1–1 mg/kg s.c.) was administered 30 min before the removal of the topical capsaicin patch, under the aforementioned conditions. Loperamide caused a robust dose-dependent prevention of capsaicin-induced alldynia. For the 38°C water condition, a two-way (time × loperamide dose) repeated measures ANOVA was significant for time \( F(4,12) = 8.57 \) and for loperamide dose \( F(3,9) = 5.02 \). In 42°C water, similar findings were obtained [time: \( F(4,12) = 10.59 \); loperamide dose: \( F(3,9) = 9.39 \)]. This dose-dependent effect could be clearly observed at the time of peak capsaicin alldynia (i.e., 30 min after the removal of the topical capsaicin patch; Fig. 2; Table 2). No obvious behavioral effects of s.c. loperamide were observable under these conditions by an experimenter experienced in behavioral rating in this species.

In separate studies, fentanyl (0.0032–0.032 mg/kg s.c.) was administered 15 min before the removal of the topical capsaicin patch. Fentanyl also caused dose-dependent prevention of alldynia (Fig. 3). In the 38°C water condition, a two-way (time × fentanyl dose) repeated measures ANOVA was significant for time \( F(4,12) = 5.75 \) and for fentanyl dose \( F(3,9) = 5.35 \). In 42°C water, similar findings were obtained [time: \( F(4,12) = 4.04 \); fentanyl dose: \( F(3,9) = 5.66 \); see also Table 2]. In one of the four subjects, the largest fentanyl dose (0.032 mg/kg) did not result in latencies ≥10 s, at the time of peak capsaicin alldynia (30 min after the removal of the capsaicin patch). A larger fentanyl dose (e.g., 0.056 mg/kg) could not be studied in this subject, due to the occurrence of respiratory depression observed in a pilot study.

**Antagonism of the Ability of Loperamide and Fentanyl to Prevent Alldynia.** In four separate experiments, the largest doses of loperamide and fentanyl used above (1 mg/kg and 0.032 mg/kg, respectively) were studied after a 30-min pretreatment with naltrexone (0.1 mg/kg s.c.) or its analog, methylnaltrexone (0.32 mg/kg s.c.). The findings are

![Fig. 1. Time course and concentration-dependence of the thermal alldynic effect of topical capsaicin (0.004 M; left panels) or OLDA (0.0013–0.004 M; right panels) in 38°C and 42°C water (upper and lower panels, respectively). All data are mean ± S.E.M. (n = 4) of one determination, except for the capsaicin and OLDA 0.004 M data, which are the mean of two determinations each. Abscissae, time in minutes from the removal of the topical patch; BL indicates baseline latencies. Ordinates, tail withdrawal latencies in seconds (20 s maximum allowed latency).](image-url)
summarized graphically at the time of peak capsaicin-induced allodynia (i.e., 30 min after removal of the topical capsaicin patch, in 42°C water) (Fig. 4). A two-way (treatment × time) repeated measures ANOVA was completed for the effects of loperamide (1 mg/kg) alone or after naltrexone or methylnaltrexone pretreatment, in 42°C water. A significant effect of treatment was found \( F(2,6) = 11.32 \), and a Dunnett’s test revealed that both naltrexone and methylnaltrexone pretreatment conditions were different from those of loperamide alone. Effects in 38°C water were not analyzed in these probe antagonism studies (the 1 mg/kg loperamide dose was supramaximal for two of four subjects with the 38°C stimulus; Fig. 2). An analogous two-way repeated measures ANOVA was completed for fentanyl (0.032 mg/kg) alone or after naltrexone or methylnaltrexone pretreatment, in 42°C water. A significant effect of time was found \( F(4,12) = 4.41 \). In a follow-up one-way repeated measures ANOVA at a standard time of peak allodynia (30 min after topical capsaicin removal), a significant effect of treatment was found \( F(2,6) = 5.22 \); Dunnett’s tests revealed that the naltrexone (0.1 mg/kg) pretreatment condition was different from that of fentanyl (0.032 mg/kg) alone. By contrast, the methylnaltrexone (0.32 mg/kg) pretreatment condition was not different from that of fentanyl alone.

Effects of Loperamide and Fentanyl in Reversing Ongoing Capsaicin-Induced Allodynia. In separate studies, the largest loperamide and fentanyl doses used above (1 mg/kg and 0.032 mg/kg; respectively) were administered immediately after the onset of robust allodynia, under the present conditions. That is, loperamide or fentanyl (or vehicle) was administered immediately after the 15-min test, after the removal of topical capsaicin (see Figs. 1 and 5). In the 38°C water condition, a two-way repeated measures ANOVA (time × treatment) narrowly missed significance \( F(2,6) = 4.54; p = 0.06 \), whereas a Dunnett’s test revealed that fentanyl (but not loperamide) was significantly different from vehicle. For the 42°C water condition, a similar two-way ANOVA was significant for treatment \( F(2,6) = 9.45 \), and a Dunnett’s test confirmed that fentanyl (but not loperamide) was significantly different from vehicle.

Effects of Loperamide and Fentanyl in Preventing Endovanilloid (OLDA)-Induced Allodynia. The largest doses of loperamide and fentanyl (1 mg/kg and 0.032 mg/kg, respectively) were also administered before topical OLDA (0.004 M), under conditions identical to those described above (Fig. 6). Given the lack of robust OLDA-induced allodynia in 38°C water (see Fig. 1), only data in 42°C water were analyzed. A two-way repeated measures ANOVA (time × treatment) revealed a significant effect of time \( F(4,12) = 8.77 \) and treatment \( F(2,6) = 7.90 \). Dunnett’s tests revealed that both loperamide and fentanyl conditions were significantly different from those of OLDA alone.

Antinociceptive Effects of Loperamide and Fentanyl. In these subjects, 48°C (in the absence of topical capsaicin) was the lowest temperature at which rapid (e.g., <3 s) and consistent tail withdrawal latencies were observed (pilot studies). Therefore, the antinociceptive effects of s.c. vehicle, loperamide, and fentanyl were studied against the 48°C thermal stimulus, to sensitively detect antinociceptive effects. In a 90-min vehicle test, preinjection latencies at 48°C had a mean of 1.8 s (S.E.M. = 0.5). Vehicle administration (s.c.; 0.1 ml/kg) was without effect on these latencies (Fig. 7). Latencies in 42°C water were at cutoff (20 s) throughout the vehicle test (not shown). Loperamide, up to the largest dose studied above (1 mg/kg), was also without effect at 48°C, whereas fentanyl (0.01–0.032 mg/kg) produced a dose-dependent antinociceptive effect. A two-way (time × fentanyl dose) ANOVA revealed a significant effect of time \( F(5,15) = 4.24 \) and an interaction between time and fentanyl dose \( F(10,30) = 2.85 \). A Dunnett’s test revealed that the larger dose of fentanyl (0.032 mg/kg) was significantly different from vehicle. A one-way repeated measures ANOVA at this fentanyl dose [for time (baseline and post-fentanyl, 0.032 mg/kg) revealed a significant effect of time \( F(6,18) = 5.98 \). Newman-Keuls tests revealed that the 30- and 45-min time points post-fentanyl were significantly different from baseline. These two time points (30 and 45 min post-fentanyl) were not different from each other.

A summary of antiallodynic and antinociceptive dose-effect curves and potency (ED\(_{10}\)- values) for loperamide and fentanyl is presented graphically in Fig. 8 and Table 2. Overall, fentanyl was more potent than loperamide as an antiallodynic agent, at either 38°C or 42°C. Fentanyl exhibited similar potency, across all the present endpoints, as an antiallodynic or antinociceptive agent. Loperamide appeared to exhibit an intensity-dependent profile as an antiallodynic agent, in that the dose-effect curve for 38°C was to the left of that for 42°C (although ED\(_{10}\)- values were not significantly different). Loperamide was ineffective as an antinociceptive agent, over this dose range.

**Discussion**

In the present studies, loperamide (0.1–1 mg/kg s.c.), acting as a peripherally selective \( \mu \)-agonist after s.c. administration, produced a prevention of topical capsaicin-induced allodynia. However, up to 1 mg/kg, loperamide was completely devoid of thermal antinociceptive effects in these sub-
jects (a presumed centrally mediated effect of μ-agonists). By comparison, the centrally penetrating μ-agonist, fentanyl, produced both antiallodynia and antinociception over a similar dose range (fentanyl potency at each of these endpoints was very similar). These findings suggest that systemically administered, peripherally selective μ-agonists may have antiallodynic effects in vivo in primates, in the relative absence of robust centrally mediated effects, such as antinociception. This is consistent with previous findings in rodents, using systemic loperamide (Takasuna et al., 1994; Reichert et al., 2001). Other studies in rodents also indicate that local (e.g., injected or topical) loperamide also produces antinociceptive effects in certain pain models (Nozaki-Taguchi and Yaksh, 1999; DeHaven-Hudkins et al., 2002; Menendez et al., 2003). Loperamide doses larger than 1 mg/kg could not be tested under the present solubility conditions. In another study with loperamide in primates, substantially larger loperamide doses (e.g., 8 mg/kg s.c.) were associated with acute toxicity.

**TABLE 2**

Antiallodynic and antinociceptive potency of loperamide and fentanyl (n = 4, unless otherwise stated)

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Antiallodynia ED_{10} (95% CL)^{a}</th>
<th>Antinociception ED_{10} (95% CL)^{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38°C</td>
<td>42°C</td>
</tr>
<tr>
<td>Loperamide</td>
<td>0.18 (0.05–0.6)</td>
<td>0.59 (0.25–1.4)</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>0.0078 (0.0014–0.042)</td>
<td>0.012 (0.0022–0.063)</td>
</tr>
</tbody>
</table>

95% CL, 95% confidence limits.

^a ED_{10} values are in mg/kg, measured at the time of peak capsaicin-induced allodynia (i.e., 30 min after topical capsaicin removal).

^b ED_{10} values were determined, for each opioid agonist, at a time equivalent to that in the allodynia studies (i.e., 60 min and 45 min after loperamide and fentanyl injection, respectively).

^c All fentanyl ED_{10} values are based on n = 3. The fourth subject did not attain the 10-s level of effect in any fentanyl condition, up to the largest fentanyl dose studied. Larger fentanyl doses could not be evaluated in this subject, due to the occurrence of respiratory depression.

Fig. 3. Prevention of topical capsaicin (0.004 M)-induced thermal allostodynia by s.c. fentanyl. Fentanyl doses are in mg/kg; all other details are as in Figs. 1 and 2.

Fig. 4. Summary of antagonism experiments with naltrexone (NTX; 0.1 mg/kg s.c.) or methylnaltrexone (MNTX; 0.32 mg/kg s.c.), to investigate sensitivity of loperamide (LOP)- or fentanyl (FEN) (1 mg/kg and 0.032 mg/kg, respectively)-induced prevention of capsaicin (CAP)-induced allodynia. Data are summarized for the 42°C thermal stimulus, at the time of peak capsaicin-induced allostodynia (30 min after removal of the topical capsaicin patch; all n = 4). Mean of one to two determinations (statistical analyses are in text). Veh, vehicle

Fig. 5. Reversal of topical capsaicin (0.004 M)-induced allostodynia by s.c. vehicle or s.c. loperamide or fentanyl (1 mg/kg and 0.032 mg/kg, respectively). In these experiments, vehicle, loperamide, or fentanyl was injected immediately after the test occurring 15 min after topical capsaicin removal.
including respiratory failure (Yanagita et al., 1979). At the largest dose used herein, loperamide did not have obvious behavioral effects, and no untoward effects were observed during or after loperamide dosing sessions.

Probe antagonist studies were completed with naltrexone and its analog, methylnaltrexone (which only poorly crosses the blood-brain barrier). Loperamide’s antiallodynic effects were sensitive to both naltrexone (0.1 mg/kg) and methylnaltrexone (0.32 mg/kg). However, the antiallodynic effects of fentanyl were sensitive to naltrexone but not methylnaltrexone. The present naltrexone dose is sufficient to antagonize diverse µ-receptor-mediated effects in this species; it is presumed that naltrexone would be able to occupy both central and peripheral µ-opioid receptor pools, following systemic administration (France et al., 1990; Ko et al., 1998a). The presently used s.c. methylnaltrexone dose is sufficient to produce a blockade of the effects of morphine on intestinal transit in humans (an effect presumed to be mediated by peripherally located µ-receptors) (Yuan et al., 2002). This methylnaltrexone dose is also sufficient to cause a blockade in a κ-opioid neuroendocrine effect in this species; this neuroendocrine effect (prolactin release) is presumed to be mediated outside the blood-brain barrier (Butelman et al., 2004). Ex vivo studies suggest that methylnaltrexone is a more potent antagonist of µ- than of κ-receptor-mediated effects (Yuan and Foss, 1999). Taken together, the above findings lead to the conclusion that the presently used methylnaltrexone dose is sufficient to cause a blockade of peripheral µ-opioid systems, under the present conditions. Both loperamide and fentanyl have binding selectivity for µ-over κ- or δ-receptors (Toll et al., 1997; DeHaven-Hudkins et al., 1999). Overall, the present antagonism studies are therefore consistent with the conclusion that loperamide and fentanyl produced their antiallodynic effects by acting predominantly at peripherally and centrally located µ-receptors, respectively.

The effects of the opioid antagonists alone were not studied herein. Under specific experimental conditions, there have been reports of opioid antagonists (e.g., naloxone) enhancing capsaicin-induced pain ratings in humans (Anderson et al., 2002). Such a potential enhancement would not have been easily detectable under the present conditions, due to the presence of maximal “floor” allodynia at several of the present time points after topical capsaicin.

Loperamide appeared to be less effective than fentanyl in reversing, rather than preventing, topical capsaicin-induced allodynia. These are the first studies in primates in which the ability of µ-agonists to prevent and reverse capsaicin-induced allodynia has been directly investigated, to our knowledge. The present probe experiments suggest that the triggering of allodynia (in this model) may be sensitive to peripheral µ-opioid effects, whereas the maintenance of ongoing allodynia may be relatively less sensitive to such effects (as evidenced by the limited ability of loperamide relative to fentanyl). Interestingly, in mice, i.p. morphine (acting through a proposed peripherally mediated opioid mechanism) was more potent in the acetic acid-induced writhing assay when ad-
ministered as a pretreatment than when administered after the onset of the writhing (Reichert et al., 2001). As mentioned above, both peripheral VR1 and μ-opioid receptor populations (e.g., in primary afferents) exhibit a substantial degree of plasticity, in animal models of neuropathic or inflammatory insults (Ji et al., 2002; Truong et al., 2003; Zollner et al., 2003). Therefore, loperamide’s present lack of effectiveness in reversing ongoing allodynia in this model does not necessarily indicate that loperamide would be ineffective in experimental pain models (or in clinical situations) with ongoing neuropathic or inflammatory pain, in which such plasticity may have occurred.

As a further caveat, loperamide (and presumably other peripherally selective μ-agonists) may have potentially undesirable effects mediated by peripheral opioid receptors. Such potential undesirable effects could include constipation, and immune and neuroendocrine effects. The “therapeutic window” for systemically administered loperamide in causing antiallodynia versus causing the aforementioned peripherally mediated undesirable effects has not been directly studied, to our knowledge.

The recently discovered endovanilloid OLDA (Chu et al., 2003) also produced concentration-dependent thermal allodynia after topical administration in primates. OLDA displayed a profile of maximum allodynia in 42°C water similar to that of capsaicin, and maximal allodynia was observed at a similar topical concentration (0.004 M) (present study; Butelman et al., 2003). In vitro, OLDA was also approximately equipotent and equieffective to capsaicin as an agonist at cloned VR1 receptors (Chu et al., 2003). Unlike other endovanilloids (e.g., anandamide, N-arachidonyl dopamine), OLDA has a relatively high degree of selectivity for VR1 receptors over CB1 cannabinoid receptors (Chu et al., 2003). Thus, OLDA may be an especially valuable tool to study the in vivo effects of endovanilloids. Locally administered OLDA caused thermal hyperalgesia in mice (Chu et al., 2003). The presently reported studies are the first data with OLDA in nonhuman primates, to our knowledge, and are consistent with a role for this endovanilloid in the process of thermal allodynia. In probe studies, loperamide and fentanyl both prevented OLDA-induced allodynia (similar to their effect on capsaicin-induced allodynia). These are, to our knowledge, the first studies on opioid blockade of OLDA-induced allodynia in any species. Overall, these initial studies suggest that endovanilloid-induced allodynia may be sensitive to the same types of opioid analgesics as capsaicin-induced allodynia (a compound widely used to model endogenous opioid states, which may potentially depend in part on endovanilloid release).

Prior in vitro and in vivo studies with experimental animals have identified complex interspecies differences in VR1 receptor pharmacology (e.g., rat versus human) (Szallas and Blumberg, 1993; Walker et al., 2003). Radioligand binding studies indicate that the in vitro pharmacology of native macaque VR1 receptor (e.g., from dorsal root ganglia) may be only modestly different from that of human VR1 receptors (we are not aware of published sources of information on cloned nonhuman primate VR1 receptors) (Szabo et al., 2002). Therefore, available data at this time suggest that maceaux may be valuable model species, due to the similarity in the in vitro VR1 receptor pharmacology with that of humans, as well as the similarity in the in vivo allodynic effects of topical capsaicin (Culp et al., 1989; Kupers et al., 1997).

Overall, the present studies show that loperamide, which acts as a peripherally selective μ-agonist after systemic administration, can prevent capsaicin-induced thermal alldynia in primates, in the absence of centrally mediated thermal antinociceptive effects. This suggests that systemically administered peripherally selective μ-agonists could potentially produce antiallodynia in the relative absence of undesirable centrally mediated effects (e.g., cognitive side effects and respiratory depression) (O’Mahony et al., 2001). Given the reported relevance of peripheral μ-opioid analgesia in a variety of rodent pain models including visceral pain, burn pain, and bone pain (Takasuna et al., 1994; Houghton et al., 1998; Nozaki-Taguchi and Yaksh, 1999; Junger et al., 2002; Menendez et al., 2003), the present studies support the hypothesis that peripherally selective μ-opioids are valuable pharmacotherapeutic targets for these painful conditions and may be a useful adjunct to current therapeutic approaches.

References


Address correspondence to: Dr. E. Butelman, Rockefeller University, Box 171, 1230 York Ave., New York, NY 10021. E-mail: butelmne@mail.rockefeller.edu