Topical Capsaicin-Induced Allodynia in Unanesthetized Primates: Pharmacological Modulation

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ABSTRACT
Topically administered capsaicin produces thermal allodynia, and this effect has been used to investigate pain transduction and its pharmacological modulation. This study investigated the parameters of topical capsaicin-induced thermal allodynia in unanesthetized rhesus monkeys and its pharmacological modulation by centrally acting compounds [a κ-opioid agonist: (5α,7α,8β)-(+)-N-methyl-N-(7-[1-pyrrolidinyl]-1-oxaspiro[4.5]dec-8-yl)-benzeneacetamide (U69,593); and noncompetitive N-methyl-D-aspartate (NMDA) antagonists: ketamine and MK-801 (dizocilpine)]. Rhesus monkeys (n = 4) were studied within the warm water tail withdrawal assay (20-s maximum latency), using thermal stimuli that are normally not noxious (38 and 42°C). Capsaicin was applied topically on the tail (0.0013 and 0.004 M capsaicin solution on a 1-cm² patch; 15-min contact). Topical capsaicin produced concentration-dependent thermal allodynia in both temperatures, robustly detected 15 to 90 min after topical capsaicin removal. A similar allodynic profile was observed with topical administration of the “endovanilloid” N-arachidonoyl-dopamine. The κ-agonist U69,593 (0.01–0.1 mg/kg, s.c.) dose dependently prevented capsaicin (0.004 M)-induced allodynia in 38 and 42°C, and the largest U69,593 dose also reversed ongoing allodynia within this model. Two NMDA antagonists, ketamine and MK-801 (0.32–1.8 and 0.032–0.056 mg/kg, respectively), also prevented capsaicin-induced allodynia in 38°C, but only variably in 42°C, at doses that did not cause robust thermal antinociceptive effects. At the largest doses studied, ketamine but not MK-801 also briefly reversed ongoing capsaicin-induced allodynia. The present model of topical capsaicin administration may be used to study antiallodynic effects of opioid and nonopioid compounds, as well as their ability to prevent and reverse allodynia, in unanesthetized nonhuman primates in the absence of tissue disruption.

The present studies investigated the parameters of topical capsaicin-induced thermal allodynia in unanesthetized nonhuman primates and the pharmacological manipulation of this allodynia by a κ-opioid agonist and by noncompetitive NMDA antagonists. Capsaicin is the main pungent compound in “hot” chili peppers; its main site of action is the vanilloid VR1 (or TRPV1) receptor (Caterina et al., 1997; Caterina and Julius, 2001). This receptor is present in many neuronal populations in brain and periphery and notably on primary afferent nociceptor pathways (e.g., C-fiber populations) (Caterina et al., 2000; Ji et al., 2002). VR1 receptors gate a cation channel that is opened in response to noxious temperatures (e.g., >46°C) or low pH. VR1 receptor function is also sensitized in the presence of mediators that may be released as a result of inflammation or tissue injury (e.g., prostaglandins, bradykinin, and substance P) (Raja et al., 1999). Thus, acute application of capsaicin, intradermally or topically, results in an enhanced afferent signaling in response to temperature increases (LaMotte et al., 1992). This results in allodynia (pain in response to normally innocuous stimuli) and hyperalgesia (an enhanced response to a painful stimulus) in experimental animals and humans (LaMotte and Campbell, 1978; Culp et al., 1989; Raja et al., 1999).

Acute injected (e.g., intradermal) and topical capsaicin have been studied extensively as experimental noxious stimuli in humans (LaMotte et al., 1992; Liu et al., 1998). Thermal allodynia as a result of injected capsaicin and its pharmacological modulation has also been studied in unanesthetized nonhuman primates (Ko and Woods, 1999; Ko et al., 1999). To our knowledge, however, the pharmacological modulation of topical capsaicin-induced allodynia has not been studied in unanesthetized nonhuman primates (Ko and Woods, 1999; Ko et al., 1999).
The present mode is also amenable to the direct comparison of allodynia prevention versus allodynia reversal by these pharmacological agents. It has been suggested that different pharmacological substrates may be used to modulate allodynia under these two conditions (Wallace et al., 2002). The present model is also amenable to the direct comparison of allodynia prevention versus allodynia reversal by these pharmacological agents. It has been suggested that different pharmacological substrates may be used to modulate allodynia under these two conditions (Winger et al., 2002). This suggests that there may be important differences in the thermal antinociceptive and antiallodynic effects of centrally-acting compounds.

We report here on the parameters of topical capsaicin-induced thermal allodynia in unanesthetized nonhuman primates, within a newly adapted model (Culp et al., 1989; Kupers et al., 1997). We further report on the pharmacological modulation of this allodynia by a centrally penetrating nonpeptidic κ-agonist, U69,593, compared with a peptidic κ-agonist, the dynorphin analog E-2078. We also studied the effects of two NMDA antagonists, ketamine and MK-801, which are known to differ in their systemic potency and duration of action (Koek et al., 1988; Beardsley et al., 1990; Winger et al., 2002). The present model is also amenable to the comparison of centrally-acting compounds. It is possible based on differences in the onset of activation at VR1 receptors (LaMotte et al., 1992). Recent studies in unanesthetized humans detected differences in the neuroanatomical centers activated by normally noxious (“suprathreshold”) thermal stimuli and by thermal allodynic stimuli (Lorenz et al., 2002). This suggests that there may be important differences in the thermal antinociceptive and antiallodynic effects of centrally-acting compounds.

Materials and Methods

Subjects

Adult, gonadally intact rhesus monkey females (Macaca mulatta; age range: 7–11 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 7:30 AM). Monkeys were fed approximately 11 jumbo primate chow biscuits (Pfizer Feeds, Richmond, VA) daily, supplemented by fruit and multivitamins. Water was freely available in home cages, via an automatic water tap. 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.

Procedures

Thermal Allodynia. The present assay is a modification of the warm water tail withdrawal assay, adapted to study topical capsaicin-induced allodynia (Ko et al., 1999). The method of topical capsaicin administration was adapted from a study characterizing its effects in a nonhuman primate and in humans (Kupers et al., 1997).

Monkeys sit in custom made polycarbonate and aluminum primate chairs within a temperature-controlled room (20°–22°C). 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 (control) latencies are determined in 38 and 42°C water stimuli. 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 and 42°C thermal stimuli are normally non-noxious in human or nonhuman primates) (Culp et al., 1989). Following baseline determination, the tail is gently dried and then exposed to an isopropyl alcohol pad. The topical capsaicin patch is then applied (as described below) for 15 min. At the end of the topica l 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, separated from each other by 2 min approximately.

Topical Capsaicin Administration. This is an adaptation of techniques previously used in humans and a nonhuman primate (Culp et al., 1989; Kupers et al., 1997). A 1-cm² patch of two-plex (Johnson & Johnson, Arlington, TX) is attached on waterproof adhesive backing (23-mm diameter; Active Strips; 3M Health Care, St. Paul, MN). This is in turn attached onto elastic adhesive tape (5 cm wide; Elastikon; Johnson & Johnson). Capsaicin (either 0.0013 or 0.004 M) is dissolved in a vehicle composed of 70% ethanol and 30% sterile water by volume, approximately 15 min before use. Capsaicin (0.3 ml of the above solution) is slowly injected onto the gauze patch, saturating the patch, and avoiding 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 a 15-min 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., 38 and 42°C). Consecutive sessions with topical capsaicin in the same subject were typically separated by 7 days.

Pharmacological Modulation of Topical Capsaicin-Induced Allodynia

In allodynia prevention studies, a single dose of a compound (e.g., U69,593, E-2078, ketamine, or MK-801) was administered as pretreatment to topical capsaicin (0.004 M). In allodynia reversal studies, a single dose of a compound was administered after capsaicin allodynia was ongoing, i.e., immediately after the 15-min tests, followed by testing at the remaining timepoints (30, 60, and 90 min), as above.

Assay of Thermal Antinociception. In separate studies, the thermal antinociceptive effects of the largest dose of U69,593, ketamine, and MK-801 were studied for comparison. The main purpose of these probe studies was: 1) to determine whether subjects were able to emit the required withdrawal response at the times that antiallodynia is observed and 2) to determine whether antiallodynic 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 50°C (noxious) thermal stimuli (Dyakstra and Woods, 1986). After baseline determination, subjects were injected with a single s.c. dose of U69,593, ketamine, or MK-801 in the scapular region. This was followed by measurement of tail withdrawal latencies at different time points, up to 120 min after injection.

Design

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

Concentration-Dependence and Local Influence of Capsaicin-Induced Allodynia and Comparison to the Endovailloid, NADA. The effects of topical vehicle and topical capsaicin (0.0013 and 0.004 M) applied on the tail were studied in separate experiments. All other topical capsaicin studies in this article used only the highest capsaicin concentration (0.004 M). The 0.004 M capsaicin concentration was studied alone in three determinations at intervals of at least 1 week through the course of these studies. A
Further control determination of capsaicin (0.004 M) allodynia was made following a systemic s.c. sterile water (i.e., vehicle) injection 15 min before capsaicin removal. In a separate control experiment, this capsaicin concentration (0.004 M) was studied after topical administration to a remote site (the calf area) under identical conditions. The purpose of this control experiment was to determine whether the observed allodynia is due to a local effect of capsaicin (i.e., on the tail), a systemic effect of capsaicin, or general behavioral disruption. The allodynic effects of the "endovanilloid" VR1 agonist NADA (0.0013 and 0.004 M) were studied after topical administration on the tail, under identical conditions (Huang et al., 2002). The effects of topical NADA were compared with a redetermination of topically administered vehicle.

Effects of U69,593. The effectiveness of U69,593 (0.01, 0.032, 0.1 mg/kg; s.c.) in preventing capsaicin-induced allodynia was studied. U69,593 was administered 15 min before the removal of topical capsaicin; two determinations were made at each U69,593 dose. In two antagonism studies, the antiallodynic effect of the largest U69,593 dose (0.1 mg/kg) was studied 30 min after pretreatment with either naltrexone (0.32 mg/kg) or methyl-naltrexone (0.32 mg/kg) followed by testing as above. A control experiment was designed to test whether apparent antiallodynic effects of U69,593 were due to an inability to detect and respond to environmental stimuli (e.g., due to sedation or motor disruption). Thus, a higher intensity noxious stimulus, 50°C, was probed 15 and 30 min after capsaicin removal (times of peak alldynia) after administration of the largest U69,593 dose (0.1 mg/kg), as described above.

In separate studies, the effectiveness of U69,593 (0.1 mg/kg; compared with vehicle) in reversing capsaicin-induced alldynia was studied. 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 U69,593, and this was followed by standard testing at the remaining standard timepoints (30, 60, and 90 min after capsaicin removal).

The thermal antinociceptive effects of the largest dose of U69,593 (0.1 mg/kg; n = 3) were studied against a 50°C stimulus, in the absence of capsaicin. Prior studies show that s.c. U69,593 causes thermal antinociception in this species (i.e., in 50°C) over the present dose range (0.01–0.1 mg/kg) (France et al., 1994; Ko et al., 1998; Butelman et al., 1999).

Effects of the Stable Dynorphin A(1-8) Analog E-2078. The effectiveness of E-2078 (0.1 or 0.32 mg/kg, i.v.; n = 3–4) in preventing capsaicin-induced alldynia was studied. E-2078 was administered i.v. 15 min before topical capsaicin removal.

Effects of Ketamine and MK-801. The effectiveness of ketamine and MK-801 (0.32–1.8 and 0.032–0.056 mg/kg, respectively) in preventing capsaicin-induced alldynia were studied as above. Pretreatment times relative to capsaicin removal were 20 and 15 min for ketamine and MK-801, respectively. The effectiveness of the largest ketamine and MK-801 doses in reversing ongoing alldynia were also studied, as described above. In pilot antinociception studies (n = 3), the antinociceptive effects of the largest dose of ketamine and MK-801 were studied in 50°C water in the absence of capsaicin treatment.

Data Analysis. Tail withdrawal latency was the dependent variable in these studies. This was obtained principally from 38 and 42°C in the presence of capsaicin (antiallodynia) or from 50°C in the absence of capsaicin (antinociception). Data are presented graphically as mean ± S.E.M. Data are analyzed in either one or two-way repeated measures ANOVAs, followed by Dunnett's tests when applicable. The level of significance (α) was set at the 0.05 level throughout.

Test Compounds. Naltrexone HCl (NIDA Drug Supply System, Baltimore MD), naltrexone methobromide (methyl-naltrexone; kindly supplied by Dr. C. S. Yuan, Dept. of Anesthesiology, University of Chicago), and MK-801 (Sigma-RBI, St. Louis, MO) were dissolved in sterile water. U69,593 (Pharmacia, Kalamazoo, MI) was dissolved in sterile water with the addition of lactic acid, to a pH of approximately 6; this stock solution was then diluted with sterile water as appropriate. E-2078 (N-methyl-Tyrβ,N-methyl-Argα,D-Leuβ,dynorphin A1-[8]ethylamide; Eisai, Ibaraki, Japan) was dissolved in sterile saline approximately 10 min before i.v. injection. Ketamine HCl (100 mg/ml stock; Ketaset, Fort Dodge, IA) was diluted with sterile water as necessary. Injections of the above compounds (s.c. in the scapular region) were made in volumes of 0.05 to 0.1 ml/kg; doses are presented in the forms of the compounds mentioned above. Capsaicin (98% pure; Sigma-Aldrich, St. Louis MO) and NADA (Tocris Cookson, Ellisville MO) were prepared in 70% ethanol/30% sterile water vehicle, approximately 15 min before topical use.

Results

Baseline Performance in the Allodynia Assay

Under baseline conditions, monkeys did not remove their tail from 38 and 42°C thermal stimuli. That is 20-s (cutoff) latencies were typically observed. For comparison, a higher temperature, 50°C, is noxious and yields baseline tail withdrawal latencies below 2 s in the present subjects (see below). The administration of topical vehicle on the tail did not cause a change in mean withdrawal latencies when tested up to 60 min after topical vehicle removal (Fig. 1).

Effects of Topical Capsaicin

Topical capsaicin (0.0013 M) did not produce robust changes in withdrawal latencies in 38 or 42°C stimuli (Fig. 1). By contrast, a 1/2 log unit higher capsaicin concentration (0.004 M) caused robust thermal alldynia in both 38 and 42°C, as detected over a standard 90-min test period (three separate determinations; n = 4 each). This effect was also observed in a further determination with capsaicin (0.004 M), following systemic s.c. sterile water pretreatment. That is, capsaicin-induced alldynia after systemic s.c. sterile water pretreatment was not significantly different from that observed in the mean of the three determinations of capsaicin alone. Two-way repeated measures [time × determination] ANOVAs were nonsignificant in 38 and 42°C for either main effects of determination or time × determination interaction. Therefore, the above four experiments with topical capsaicin (0.004 M) were averaged for statistical analyses and are presented graphically. Overall, two-way repeated measures ANOVAs (time × capsaicin concentration) revealed a significant effect of capsaicin concentration on latencies in 38°C [F(2,6) = 184.4] and 42°C [F(2,6) = 187.2] in the 60 min following capsaicin removal.

In 38°C water, an apparent peak alldynic effect was detected at the 30-min time point after topical capsaicin (0.004 M) removal; latencies partially returned to baseline levels by the end of the 90-min period. Robust alldynic effects in the higher temperature (42°C) could be detected from 15 min onwards, with an apparent "floor effect" observed up to 60 min. Only a modest return to precapsaicin latencies was detected by the end of the 90-min test in 42°C. The alldynic effects of topical capsaicin (0.004 M) had completely dissipated by 24 h (one determination, n = 4). Based on the observed time course of alldynia, 30-min postcapsaicin removal was selected for antiallodynic dose-effect curve analyses (see below; Table 1).

In a separate experiment, capsaicin (0.004 M) was applied topically in the calf area of the leg, followed by tail with-
Under these conditions, thermal allodynia was not detected. For example, at 30 min after topical capsaicin removal from the leg, all subjects had a 20-s tail withdrawal latencies in both 38 and 42°C (not shown).

Effects of Topical NADA

The endovanilloid NADA (0.0013 and 0.004 M, compared with a vehicle redetermination) also displayed concentration-dependent thermal allodynia after topical administration on the tail (Fig. 1). In particular, floor effects were observed in 42°C between 15 and 90 min after removal of NADA (0.004 M). Overall, two-way repeated measures ANOVAs (time × NADA concentration) revealed a significant effect of NADA concentration on latencies in 38°C [F(2,6) = 110.82] and 42°C [F(2,6) = 488.35] in the 90 min following NADA removal. The allodynic effects of NADA in both 38 and 42°C had fully dissipated by 24 h (not shown).

Effects of U69,593 Treatment on Capsaicin-Induced Allodynia

Prevention of Allodynia. U69,593 (0.01–0.1 mg/kg, s.c.; two determinations at each dose) was administered as a s.c. bolus injection in the midscapular region 15 min before the removal of topical capsaicin (0.004 M). U69,593 caused a prevention of allodynia in 38 and 42°C (Fig. 2). This antiallodynic effect was evident at peak allodynia times (e.g., 30 min after capsaicin removal) and had dissipated by 90 min after capsaicin removal. Two-way repeated measures ANOVAs (time × treatment; i.e., capsaicin alone or U69,593 dose) revealed a significant interaction between time and treatment in 38°C [F(12,36) = 2.93]. A similar interaction was detected in 42°C [F(12,36) = 4.53], as well as a main effect of treatment [F(3,9) = 5.57]. Dose-effect data for U69,593 are presented at a time of peak allodynia (30 min after capsaicin removal; Table 1). One-way repeated measures ANOVAs were significant for U69,593 dose [38°C: F(3,9) = 7.74; 42°C: F(3,9) = 6.39]. Dunnett’s tests revealed that the largest U69,593 dose (0.1 mg/kg) was significantly different from capsaicin alone in both temperatures.

A study was instituted to determine whether subjects could perform the required tail withdrawal response in these studies, in the presence of the largest U69,593 dose used.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Mean Latency 38°C (S.E.M.)</th>
<th>Mean Latency 42°C (S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsaicin baseline</td>
<td>2.6 (0.6)</td>
<td>1.7 (0.3)</td>
</tr>
<tr>
<td>+ U69,593 0.01</td>
<td>11.4 (3.5)</td>
<td>5.2 (1.9)</td>
</tr>
<tr>
<td>0.032</td>
<td>11.3 (3.8)</td>
<td>9.8 (4.0)</td>
</tr>
<tr>
<td>0.1</td>
<td>18.7 (1.3)</td>
<td>15.2 (2.8)</td>
</tr>
<tr>
<td>0.1 + 0.32 QNTX</td>
<td>6.6 (4.5)</td>
<td>1.5 (0.5)</td>
</tr>
<tr>
<td>0.1 + 0.32 NTX</td>
<td>20.0 (0)</td>
<td>16.0 (2.5)</td>
</tr>
<tr>
<td>+ E-2078 0.1</td>
<td>1.9 (0.04)</td>
<td>1.9 (0.5)</td>
</tr>
<tr>
<td>0.32</td>
<td>1.6 (0.2)</td>
<td>1.5 (0.4)</td>
</tr>
<tr>
<td>+ ketamine 0.32</td>
<td>1.5 (0.5)</td>
<td>0.6 (0.1)</td>
</tr>
<tr>
<td>1.0</td>
<td>15.4 (4.7)</td>
<td>2.7 (0.9)</td>
</tr>
<tr>
<td>1.8</td>
<td>10.7 (5.4)</td>
<td>7.5 (4.5)</td>
</tr>
<tr>
<td>+ MK-801 0.032</td>
<td>1.4 (0.5)</td>
<td>0.9 (0.1)</td>
</tr>
<tr>
<td>0.056</td>
<td>19.3 (0.7)</td>
<td>3.5 (1.3)</td>
</tr>
</tbody>
</table>

NTX, naltrexone; QNTX, quaternary naltrexone.

* All doses in milligrams per kilogram
U69,593 (0.1 mg/kg) was therefore administered under identical prevention conditions to those described above. In this study, however, a more intense (50°C) stimulus was also studied at times of peak allodynia (i.e., 15 and 30 min after capsaicin removal). Under these conditions, U69,593 (0.1 mg/kg) caused robust antiallodynia in 42°C (i.e., mean latency of 17.9 s; S.E.M. = 2.1, at 30 min) as expected. At this time, however, all subjects emitted rapid withdrawal responses from 50°C water in the presence of topical capsaicin (i.e., mean latency of 2.7 s; S.E.M. = 1.1; significant t test for latencies in 42 versus 50°C: t[3] = 8.54). A similar profile was observed at the earlier time point (15 min after capsaicin removal; not shown).

In an antagonism experiment, the largest dose of U69,593 (0.1 mg/kg) was also studied following 30-min pretreatment with naltrexone (0.32 mg/kg, s.c.). This pretreatment blocked the antiallodynic effect of U69,593 alone (0.1 mg/kg), both in 38°C and 42°C (Table 1). Two-way ANOVAs (time \times condition) were significant for condition in 38°C [F(1,3) = 25.84] and 42°C [F(1,3) = 87.47]. In a separate experiment, the peripherally selective antagonist quaternary naltrexone (0.32 mg/kg, s.c.) was also studied as a pretreatment to U69,593 (0.1 mg/kg) under identical conditions. This quaternary naltrexone pretreatment did not antagonize the antiallodynic effects of U69,593 (Table 1).

**Reversal of Alloodynia.** U69,593 caused a partial reversal of allodynia, most robustly observed at the 30-min time point (Fig. 3). A two-way ANOVA for reversal treatment (i.e., vehicle or U69,593) \times temperature yielded a significant effect of treatment at 30 min [F(1,3) = 26.71]. Similar analyses at the 60- and 90-min time points were not significant after this U69,593 reversal treatment.

**Antinociceptive Effects of U69,593.** Baseline latencies in 50°C water in the absence of capsaicin were rapid (e.g., in a vehicle experiment, mean baseline was 1.3 s; S.E.M. = 0.2; n = 3). Vehicle administration, s.c., did not affect latencies; for example, the highest mean value over a 60-min time course was 3.4 s (S.E.M. = 1.1). The thermal antinociceptive effects of the largest U69,593 dose used herein (0.1 mg/kg) were studied in 50°C water in the absence of topical capsaicin (n = 3). At this dose, U69,593 caused a partial antinociceptive effect in 50°C water (5–90 min after administration). The maximum mean antinociceptive effect for U69,593 (0.1 mg/kg) was observed 45 min after administration (mean = 8.2 s; S.E.M. = 5.9). This post-U69,593 time point (45 min) coincides with the time point at which the antiallodynic dose-effect curve of U69,593 is presented (see Table 1).

**Effects of E-2078 on Capsaicin-Induced Allodynia.** E-2078 (0.1–0.32 mg/kg, i.v.) did not prevent capsaicin-induced allodynia under the present conditions (5–90 min after capsaicin removal). For example, peak allodynic effects of topical capsaicin were not changed in the presence of E-2078 (Table 1).

**Effects of Ketamine on Capsaicin-Induced Allodynia.** Ketamine (0.32–1.8 mg/kg) administered 20 min before topical capsaicin (0.004 M) removal caused a partial prevention of allodynia in 38 and 42°C (Fig. 4). Thus, in two-way ANOVAs (time \times treatment; capsaicin alone or ketamine dose), a main effect of treatment was detected in 38°C [F(3,9) = 5.07] and in 42°C [F(3,9) = 4.64]. It should be noted that robust antiallodynic effects of ketamine were more clearly observed in 38 than 42°C, through the course of the session. For example, at a time of peak allodynia (e.g., 30 min after capsaicin removal) a one-way ANOVA (capsaicin alone or ketamine dose) was significant in 38°C [F(3,9) = 4.48] but not in 42°C [F(3,9) = 1.73; not significant] (see Table 1). Dunnett’s tests revealed that the
Ketamine doses are in milligrams per kilogram; all other details as in Fig. different from capsaicin alone, in 38°C. Intermediate ketamine dose only (1 mg/kg) was significantly different from capsaicin (0.004 M) alone in 38°C (Fig. 4; see also below for ketamine antinociception data).

**Reversal of allodynia.** The largest dose of ketamine used above (1.8 mg/kg) was administered in a reversal experiment, immediately after the 15 min allodynia tests (as described above). This dose of ketamine caused a brief reversal of capsaicin-induced allodynia, most robustly detected at the 30 min time point (Fig. 3). A two-way ANOVA for reversal treatment (i.e., vehicle or ketamine) x temperature yielded a significant effect of treatment at 30 min [F(1,3) = 19.46]; similar analyses at the 60 and 90 min time points were not significant.

**Antinociceptive effects of ketamine.** The thermal antinociceptive effects of the largest ketamine dose used herein (1.8 mg/kg) were studied in 50°C water in the absence of topical capsaicin (n = 3). At this dose, ketamine caused only a slight and brief antinociceptive effect in 50°C water (5–90 min after administration). The maximum mean antinociceptive effect for ketamine (1.8 mg/kg) was observed 5 min after administration (mean = 4.4 s; S.E.M. = 2.8). Fifty minutes after ketamine (1.8 mg/kg) administration (i.e., at the same time as the antiallodynia dose-effect curves; see Table 1), mean withdrawal latency from 50°C water was 1 s (S.E.M. = 0.03). Therefore, subjects could perform a rapid tail withdrawal response after the largest ketamine dose used in these studies (1.8 mg/kg), in the presence of a normally noxious thermal stimulus.

**Effects of MK-801 on capsaicin-induced allodynia.**

**Prevention of allodynia.** MK-801 (0.032–0.056 mg/kg, s.c.) was administered s.c. 15 min before the removal of topical capsaicin (0.004 M). Under these conditions, MK-801 produced a robust prevention of allodynia in 38°C (observable at the larger MK-801 dose; Fig. 5). A 2-way repeated measures ANOVA (time x treatment) revealed a significant effect of MK-801 treatment [F(2,6) = 8.93] in 38°C. A significant effect of MK-801 treatment was also detected at the 42°C stimulus [F(2,6) = 13.13]. However, the anti-allodynic effect of MK-801 appeared more variable over the 90 min test in 42°C. At these MK-801 doses (0.032 and 0.056 mg/kg), no motor effects were observable upon monkeys' return to their cages, at the end of the experiments. A larger MK-801 dose (0.1 mg/kg, s.c.) was initially studied (n = 2). However, motor disruptions were observed upon subjects' return to their cages. Thus, this MK-801 dose (0.1 mg/kg) was not studied further. The effects of MK-801 were studied at a time of peak allodynia (e.g., 30 min after capsaicin removal; Table 1). One-way repeated measures ANOVAs detected a significant effect of MK-801 dose in 38°C [F(2,6) = 240.2] but not in 42°C [F(2,6) = 2.83; not significant]. Dunnett's tests revealed that the larger MK-801 dose (0.056 mg/kg) was significantly different from capsaicin (0.004 M) alone, in 38°C.

**Reversal of allodynia.** The largest dose of MK-801 used above (0.056 mg/kg) was administered in a reversal experiment immediately after the 15-min allodynia tests (as described above). This dose of MK-801 caused a partial reversal of allodynia in two of four subjects, observable at 60 and 90 min after capsaicin removal (Fig. 3). Nevertheless, two-way ANOVAs (reversal treatment x temperature) did not yield any significant effects at either 30, 60, or 90 min.

**Antinociceptive Effects of MK-801.** The thermal antinociceptive effects of the largest MK-801 dose used herein (0.056 mg/kg) were studied in 50°C water in the absence of topical capsaicin (n = 3; 120 min test). At this dose, MK-801 caused a partial antinociceptive effect in 50°C water. Forty-five min after administration (i.e., at the same time as in the antiallodynia dose-effect data presented on Table 1), mean withdrawal latency from 50°C water in the absence of cap-
Capsaicin was 2.4 s (S.E.M. = 1.1). The peak antinociceptive effect of MK-801 (0.056 mg/kg) was observed 60 min after administration (mean = 7.9 s; S.E.M. = 6).

Discussion

These studies characterized topical capsaicin-induced thermal allodynia in unanesthetized primates and its pharmacological modulation by a centrally penetrating κ-agonist and noncompetitive NMDA antagonists (Sang, 2000). Topical capsaicin caused concentration-dependent allodynia within the tail withdrawal assay, using stimuli that are not normally noxious in primates (i.e., 38 and 42°C) (Culp et al., 1989; Kupers et al., 1997). The effect of the presently used capsaicin concentrations is consistent with previous reports of thermal allosthenia in humans, using similar administration techniques (Culp et al., 1989; Kupers et al., 1997). This suggests that there is pharmacological validity in the effects of topical capsaicin in this nonhuman primate model compared with those observed in humans. Topical administration of 0.004 M capsaicin at a remote site (the leg) did not result in thermal allodynia in the tail withdrawal assay. Therefore the presently detected allodynia is due to a local allodynic effect of capsaicin.

Larger concentrations of capsaicin have been used topically in humans (Culp et al., 1989; Anderson et al., 2002). Nevertheless, such higher concentrations would have not been informative in this model because at peak allodynia times a “floor” effect was seen in withdrawal latencies. No lesions or swelling were observable at any time during these experiments. Also, baseline latencies in 38 and 42°C did not change over the course of these experiments. This suggests that this topical capsaicin model may be practically used for pharmacological studies in primates in the absence of observable physical or behavioral changes.

Administration of topical capsaicin alone (0.004 M), interspersed through the course of these studies, did not reveal a decrease in allosthenia over time. That is, no desensitization was evident under the present frequency of capsaicin administration (typically at weekly intervals). Overall, a relatively stable baseline of allodynia could be therefore assumed to occur in the pharmacological challenge studies (see below). Previous reports support the conclusion that desensitization is observed after higher frequency capsaicin administration (Craft and Porreca, 1992; Nolano et al., 1999).

The recently discovered endogenous VR1 agonist NADA (an “endovanilloid”) also produced robust concentration-dependent thermal allosthenia, following topical administration in this model (Huang et al., 2002). The potency and effectiveness of NADA were similar to those of capsaicin within this model. Interestingly, NADA and capsaicin also displayed similar potency in vitro at VR1 receptors (Huang et al., 2002; Chu et al., 2003). NADA also has affinity at cannabinoid CB1 receptors, albeit with a high $K_i$ (0.5 μM) (Chu et al., 2003). Therefore, it may not be excluded that NADA’s present allosthenic effect was due to an interaction at CB1 receptors. However exogenous CB1 agonists produce antiallodynic or antihyperalgesic effects when administered locally in primates or rodents (Ko and Woods, 1999; Fox et al., 2001). Taken together, these data are consistent with an allodynic effect of NADA through local VR1 receptors in these studies.

These are, to our knowledge, the first studies on the allodynic effects of the endovanilloid NADA in primates.

Based on the above findings and consistent with a previous determination in a primate (Kupers et al., 1997), a topical capsaicin regimen (0.004 M exposure for 15 min) was selected for pharmacological manipulation studies. The high efficacy κ-agonist U69,593 (Remmers et al., 1999) prevented the thermal allodynic effects of capsaicin in both 38 and 42°C. The s.c. dose range over which U69,593 exhibits antiallodynic effectiveness in this assay is similar to the dose range that produces thermal antinociception (i.e., against a normally noxious 50°C stimulus in the absence of capsaicin) (France et al., 1994; Ko et al., 1998; Butelman et al., 1999). Centrally penetrating κ-agonists such as U69,593 also produce sedative and postural effects in rhesus monkeys over a similar dose range to that used herein (Butelman et al., 2001; Butelman and Kreek, 2001). A probe study was conducted at peak times of capsaicin-induced allosthenia in the presence of the largest U69,593 dose studied herein (0.1 mg/kg). This study revealed that all subjects were able to rapidly emit withdrawal responses from the supraphreshold 50°C stimulus, in the presence of capsaicin, at times when robust antiallodynia in 42°C was observed. This indicates that this antiallodynic effect of U69,593 is not due to subjects’ inability to detect a thermal stimulus per se or to emit a rapid withdrawal response. The 50°C stimulus was only studied in these probe studies to avoid adaptations that may occur when a normally noxious (supraphreshold) stimulus is superimposed upon ongoing capsaicin-induced allosthenia (Tominaga et al., 1998; Ji et al., 2002).

The antiallodynia caused by the largest dose of U69,593 (0.1 mg/kg) was blocked by pretreatment with naltrexone (0.32 mg/kg). This naltrexone dose was sufficient to block κ-receptor mediated effects in this species (Ko et al., 1998; Butelman et al., 2003). In contrast, the peripherally selective antagonist quaternary naltrexone (0.32 mg/kg) was ineffective in blocking the antiallodynic effect of U69,593. This quaternary naltrexone dose previously blocked the prolactin-releasing effects of U69,593 in this species (Butelman et al., 2003). The prolactin-releasing effects of κ-opioids are thought to be mediated by hypothalamic receptors functionally located outside the blood-brain barrier (Merchenthaler, 1991; Moore and Lookingland, 1995; Butelman et al., 2003). Taken together, these experiments suggest that the antiallodynic effects of s.c. U69,593 are mediated predominantly by centrally located κ-receptors.

The effects of naltrexone alone were not tested within these studies. Under specific conditions, opioid antagonists (e.g., naltrexone) may exacerbate capsaicin-induced effects in humans (Anderson et al., 2002). It should be noted that such a potential effect of naltrexone would not have been easily detectable in these studies at peak allodynia times, due to the presence of a floor effect in withdrawal latencies.

The present model was also amenable to the study of a compound’s ability to reverse rather than prevent allodynia (Richardson et al., 1998; Wallace et al., 2002). Thus, the largest dose of U69,593 (0.1 mg/kg) could partially reverse ongoing thermal allosthenia. This effect of U69,593 was short-lived. That is, allosthenia reversal was only robustly observable approximately 15 min after U69,593 administration. It has been suggested that prevention and reversal of chemogenic pain (including allosthenia) may be subserved by differ-
ent neurobiological substrates (Wallace et al., 2002; Yamamoto and Yaksh, 1992). These may be the first studies to examine the pharmacological reversal of topical capsaicin-induced allodynia in unanesthetized nonhuman primates.

The stable dynorphin A(1-8) analog E-2078 (0.1–0.32 mg/kg, i.v.) did not prevent capsaicin-induced allodynia, under these conditions. These i.v. doses of E-2078 are approximately 10-fold larger than doses required to produce a robust neuroendocrine effect in this species (prolactin release) over a similar time period (Butelman et al., 2003). This neuroendocrine effect of E-2078 was sensitive to quaternary naltrexone (0.32 mg/kg) and may therefore be mediated by hypothalamic κ-receptors located outside the blood-brain barrier. The lack of effectiveness of systemically administered E-2078 (mol wt. = 1036.25) may be potentially due to a lack of access to presumed epidermal or dermal sites mediating allodynia under the present conditions.

The effects of two noncompetitive NMDA antagonists, ketamine and MK-801, were also studied in this model. These compounds differ in in vivo potency and in duration of action (France et al., 1989; Beardsley et al., 1990; Winger et al., 2002). Up to the maximum dose that could be administered while avoiding motor disruptions, ketamine caused a partial prevention of thermal allodynia in 38°C and an observable effect in 42°C at early postcapsaicin times. The limited effect of ketamine in 42°C could have been a consequence of the selection of pretreatment time relative to capsaicin, in view of ketamine’s short duration of action (Winger et al., 2002). Nevertheless, the longer-lasting NMDA antagonist MK-801 also produced a robust prevention of capsaicin-induced allodynia in 38°C, but not in 42°C, at the largest dose that could be studied in the absence of motor effects (Beardsley et al., 1990; Winger et al., 2002).

Reversal studies were instituted with ketamine and MK-801. Ketamine, at the largest dose studied (1.8 mg/kg) caused a reversal of allodynia, and this effect was detectable at the earliest tested time point (i.e., approximately 15 min after ketamine injection), consistent with ketamine’s fast onset (Winger et al., 2002). By contrast, MK-801 only caused a partial and nonsignificant reversal at later time points.

At the largest ketamine and MK-801 doses used herein and at the time of peak anti-allodynia, these compounds did not have robust thermal antinociceptive effects (i.e., against a 50°C stimulus in the absence of capsaicin). This is consistent with a previous determination of the potency of these compounds in the thermal antinoceision assay (France et al., 1989). Taken together, these findings suggest that the present antiallodynic effects of ketamine and MK-801 are not due to sensorimotor deficits (e.g., inability to detect a thermal stimulus or to rapidly emit the required escape response). It should be noted that the effective antiallodynic doses of ketamine and MK-801 are the approximate maximum doses that could be administered acutely without motor disruption. Also, ketamine’s and MK-801’s antiallodynic effectiveness may be limited under these conditions (e.g., in 42°C). These findings are consistent with a narrow “therapeutic window” for ketamine-induced analgesia or antihyperalgesia in humans (Sang, 2000). These are, to our knowledge, the first studies to test the antiallodynic effects of ketamine or MK-801 in unanesthetized nonhuman primates.

These studies characterized concentration-dependent thermal allodynia after topical capsaicin administration in unanesthetized rhesus monkeys. These studies indicate that a centrally penetrating κ-agonist and different NMDA antagonists produced antiallodynia in this model (albeit with different maximum effectiveness) and that these effects were not secondary to sedative or sensorimotor effects of these compounds. Furthermore, the present studies illustrate the potential of this model for studies of antiallodynia prevention versus allodynia reversal within a single assay (Wallace et al., 2002).

References


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