Early Nociceptive Events Influence the Temporal Profile, but not the Magnitude, of the Tonic Response to Subcutaneous Formalin: Effects with Remifentanil

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ABSTRACT

Injection of dilute formalin into the hindpaw produces brief (phase 1) and persistent (phase 2) nociceptive responses in the rat. We recently reported that ongoing peripheral nerve input is required for the expression of behavioral and cardiovascular responses during phase 2. Here we evaluated the contribution of central and peripheral sensitization mechanisms, generated during phase 1, to the magnitude and temporal profile of phase 2. During phase 1, we administered analgesic doses of an ultrashort-acting opioid, remifentanil (i.v. administration from 0–5 min after 5.0% formalin injection), or anesthetic concentrations of halothane (2.1%). Inhibition of phase 1 did not reduce the magnitude of flinching and cardiovascular responses during phase 2, but it did delay their onset and/or termination. Longer remifentanil infusions (0–15 or 0–30 min) produced even longer delays (up to 30 min) in the onset and termination of flinching during phase 2; however, when remifentanil was administered during the early part of phase 2 (15–30 or 15–45 min), it did not prolong the time to termination of phase 2. Continuous infusion (10 mg/kg/hr i.v.) of a peripherally acting opiate antagonist, naloxone methiodide, did not reduce the antinociception produced by remifentanil during phase 1 but almost completely reversed the delay in the onset and termination of phase 2. We conclude that central sensitization mechanisms during phase 1 do not influence the magnitude of phase 2. We also hypothesize that remifentanil interacts with peripheral opioid receptors to impede the formalin-evoked synthesis and/or release of proinflammatory compounds during phase 1 and thus delay phase 2.

Tissue injury evokes prolonged prostaglandin- and N-methyl-D-aspartate -mediated decreases in the threshold of peripheral afferent nociceptive terminals and dorsal horn neurons, respectively (Woolf, 1983; Haley et al., 1990). These peripheral and central sensitization mechanisms influence the development of lowered pain threshold (allodynia), exacerbated pain responses (hyperalgesia) and persistent pain (Coderre et al., 1993; Woolf and Chong, 1993; Cervero, 1995). In one popular model of persistent pain in the rat, intraplantar injection of dilute formalin produces rapid-onset, short-lived (phase 1) and then persistent (phase 2) nociceptive responses. The latter may be driven by both peripheral and central sensitization mechanisms (Dubuisson and Dennis, 1977; Tjolsen et al., 1992). We recently reported that injection of a non-CNS-penetrating, quaternary local anesthetic, QX-314, into the formalin-injected hindpaw (but not the contralateral paw) completely inhibited nociceptive behaviors and blood pressure increases during phase 2 (Taylor et al., 1995a) and significantly decreased c-fos expression in the lumbar dorsal horn of the spinal cord (Taylor et al., 1995b), a result that indicates an essential contribution of ongoing peripheral nerve activity to these responses.

In the present studies, we evaluated the contribution of nociceptive processing during phase 1 to the persistent pain that characterizes phase 2, an issue that remains controversial. For example, intrathecal opioid antinociception that was restricted to phase 1 inhibited dorsal horn neuronal activity (Dickenson and Sullivan, 1987) and pain behavior (Abram and Yaksh, 1993; O’Connor and Abram, 1994) during phase 2, which indicates that central sensitization during phase 1 influences phase 2. On the other hand, local anesthetic blockade with lidocaine during phase 1 did not change dorsal horn neuronal activity (Haley et al., 1990) or pain behavior (Dalle et al., 1995) during phase 2, which indicates that neuronal activity during phase 1 is not a prerequisite for phase 2. Because these experiments were terminated 60 min after formalin injection (i.e., before phase 2 responses had subsided), one possible explanation for the discrepancy is that local anesthesia and/or opioid antinociception during Phase 1 delayed, rather than inhibited, Phase 2. Indeed, after opioid antinociception during phase 1, dorsal horn neuronal activity

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ABBREVIATIONS: MAP, mean arterial pressure; bpm, beats per minute; ANOVA, analysis of variance.
and flinching behavior rose throughout phase 2 (Dickenson and Sullivan, 1987; Abram and Yaksh, 1993) and may have continued to rise well beyond the observation period.

Therefore, to address the hypothesis that neuronal activity during phase 1 influences the temporal profile of phase 2, we inhibited nociceptive processing during phase 1 and then observed nociceptive responses until phase 2 had subsided. We not only monitored formalin-evoked pain behavior but also evaluated cardiovascular responses, both of which are reliable and objective measures of the central transmission of nociceptive signals (Taylor et al., 1995a). Rather than restrict the duration of opioid antinociception with an opiate antagonist, as in previous studies (Dickenson and Sullivan, 1987; Abram and Yaksh, 1993; Abram and Olson, 1994; O’Connor and Abram, 1994), we blocked nociceptive processing during phase 1 with a new, ultrashort-acting mu opioid analgesic, remifentanil (Feldman et al., 1991; Glass, 1995), which was recently shown to have an equilibration half-life of less than 2 min in the rat (Haidar et al., 1996).

**Materials and Methods**

**Animals**

Male albino Sprague-Dawley rats, weighing 270 to 330 g, were obtained from Charles River Laboratories (Hollister, CA). Several days before surgery, animals were individually housed in standard clear plastic cages in a temperature-controlled room (20°C ± 1°C) on a 12-hr light-dark cycle (6 A.M. lights on), with food and water provided *ad libitum*. The Committee on Animal Research of the University of California San Francisco approved all protocols.

**Arterial and Venous Catheterization**

Venous jugular catheters were constructed by heat-fusing a 3.2-cm length of PE-10 polyethylene tubing to a 7-cm length of PE-50 tubing. Under pentobarbital anesthesia (50–60 mg/kg), the external jugular vein was isolated by blunt dissection. Through a small slit cut into the vessel, the catheter, prefilled with 100 IU/ml heparin, was advanced proximally so that its tip slightly extended into the right atrium. After securing of the catheter to the vessel with 4–0 suture, the PE-50 end of the catheter was tunneled under the skin, exteriorized at the nape and sutured to the dorsal neck muscles (splenius cervicis). In some animals, chronic catheters were also placed into the left femoral artery for chronic measurement of MAP and HR, as previously described (Taylor et al., 1995a). After recovery from anesthesia, animals were returned to their cages and allowed to recover for 3 to 5 days before testing.

**Thermal Paw Withdrawal Test**

After the venous catheter was connected to PE-50 tubing containing remifentanil or saline, five or six rats at a time were acclimated to an 8 in. × 8 in. × 8 in. clear Plexiglas box on a glass floor for at least 1 hr. Absorbent paper towels, initially placed under the animal, were removed at least 30 min before testing. To facilitate acclimation and response reliability, fluctuations in room noise, vibration and temperature (72°C) were minimized. As initially described by Hargreaves et al. (1988), the thermal stimulus consisted of a radiant heat source (S-V, 50-W lamp) positioned under the glass floor directly beneath the hindpaw. When triggered, a timer was activated, and light passed through a small aperture at the top of a movable case. A photoelectric cell detected light reflected from the paw. When movement of the paw disrupted this light, the light and electronic timer turned off automatically. Voltage intensity was adjusted so that the average latency to paw withdrawal was 10 ± 0.5 sec. The experiment was performed, with this voltage intensity, 1 day later. At specified time-points, the stimulus was applied to the right paw and then repeated on the left paw. The average of the paired measurements was calculated. If the rat did not respond within 20 sec, the heat was discontinued to prevent tissue damage.

The first experiment evaluated the antinociceptive effect of a bolus injection of remifentanil. After obtaining at least four baseline latency values, we injected 100 μl of remifentanil (3 μg/kg, n = 6; 10 μg/kg, n = 10; 30 μg/kg, n = 10; or 90 μg/kg, n = 10) and evaluated paw withdrawal latency for 9 min.

The second experiment evaluated the effects of a longer remifentanil treatment. A bolus (100 μl i.v.) of saline or remifentanil (90 μg/kg) was administered. This was followed 90 sec later by a continuous infusion (25 μl/min) of saline or remifentanil (45 μg/kg/min) for 4 min. Withdrawal latency was evaluated for the first 9 min after remifentanil (n = 6), or to avoid thermal tissue damage, testing was initiated 10 min after saline (n = 5) or remifentanil (n = 5). In these latter groups, testing was continued for an additional hour.

**Formalin Test**

Because adaptation to the test environment decreases the variability associated with behavioral measurement in the formalin test (Tjolsen et al., 1992), each animal was transferred to a bedded 10 in. × 10 in. × 10 in. Plexiglas box in the laboratory, with food and water provided *ad libitum*, at least 16 hr before testing. After this acclimation period, the arterial and/or venous catheters were connected via PE-50 tubing to a pressure transducer (Kobe, Arvada, CO) and/or an infusion pump (CMA/100, Carnegie-Mellon, Stockholm, Sweden), respectively. Cardiovascular recording was begun at least 20 min later; this time period allows MAP and HR to return to a resting state after the sympathetically activating production by handling (Taylor et al., 1994). Next, animals received a 50-μl s.c. injection of either saline or formalin (37%, w/w, formaldehyde, diluted to 5.0% in 0.9% saline) into the plantar surface of the right hindpaw. In addition to MAP and HR, flinching behavior was recorded. To quantify flinching behavior during phase 1, we counted the flinches during the second and third minute after injection. After a 5-min pause, flinches were counted for 2 min at 5-min intervals. These numbers were divided by 2 to yield flinches per minute. With this method, behavior was simultaneously recorded in two animals at 1 to 2, 2 to 3, 8 to 10, 13 to 15,..., 88 to 90 min after formalin injection. Each animal was used only once, i.e., formalin injection was never repeated in the same animal.

**Infusion of remifentanil**

In all animals, a bolus (100 μl i.v.) of saline or remifentanil (90 μg/kg) was administered. This was followed 90 sec later by a continuous infusion (25 μl/min) of saline or remifentanil (45 μg/kg/min). The bolus of formalin administration and the duration of infusion were varied in three separate experiments involving eight groups (n = 8 in each group). In the first experiment, the bolus of saline or remifentanil was administered 15 sec before formalin injection, and the infusion lasted 4 min (designated “0–5 min,” the duration of phase 1). In the second experiment, the bolus of saline or remifentanil was administered 15 sec before formalin injection, and the infusion lasted 13.5 min (designated “0–15 min,” the duration of phase 1 plus the interface that precedes phase 2); in another group, the bolus of remifentanil was administered 15 min after formalin injection, and the infusion lasted 13.5 min (designated “15–30 min,” the first part of phase 2). In the third experiment, the bolus of saline or remifentanil was administered 15 sec before formalin injection, and the infusion lasted 28.5 min (designated “0–30 min”) in another group, the bolus of remifentanil was administered 15 min after formalin injection, and the infusion lasted 28.5 min (designated “15–45 min”).

**Infusion of remifentanil and naloxone methiodide**

To determine the peripheral opioidergic effects of remifentanil, naloxone methiodide (10.0 mg/kg/hr) was infused 30 min before formalin injection; this was continued throughout the duration of the experiment. Either saline (n = 8) or remifentanil (n = 8) was administered as in the “0–15 min” groups described above.
Administration of halothane during phase 1. Because inhalation anesthetics block flinching and cardiovascular responses during phase 1, animals were anesthetized with halothane during phase 1. After five sequential base-line measurements of MAP and HR were obtained, the animal was anesthetized with 2.1% halothane; we previously demonstrated that this concentration completely inhibited cardiovascular responses to hindpaw formalin (Taylor et al., 1995a). Saline \((n = 8)\) or 5% formalin \((n = 13)\) was then injected s.c. Three minutes after formalin injection, the halothane concentration was reduced to 1.3% to minimize rebound increases in MAP and HR. Ten minutes after injection, the halothane administration was discontinued and the animal was returned to its home cage. MAP and HR were recorded for an additional 65 min.

Data Analysis and Statistics

After the animals had acclimated to the test environment, five sequential steady-state base-line cardiovascular values were recorded. The average of these base-line values was then subtracted from each poststimulus value to yield changes in MAP or HR at each of the 70 min after formalin injection. Formalin-evoked flinching and cardiovascular responses were analyzed by two-way repeated-measures ANOVA, with Group as the between-subjects variable and Time as the repeated measure. Effects during phase 1 (minutes 1–5), interphase (minutes 11–15) and phase 2 (minutes 20–85/90) were separately evaluated. If the effects proved significant, these analyses were followed by post-hoc tests. Because the two saline control groups for the 0 to 15-min and 0 to 30-min remifentanil infusions were not different \((P > .05)\), they were combined for subsequent statistical comparisons.

Materials

Remifentanil (hydrochloride salt of 3-[4-methoxy-carbonyl-4-[(1-oxopropyl)phenylamino]-1-piperidine] propanoic acid, methyl ester; GI87084B), was kindly provided by Glaxo Research Institute (Research Triangle Park, NC) and was diluted in 0.9% isotonic saline (Baxter Healthcare Corp., Deerfield, IL). Stock solutions of formalin (aqueous solution of 37%, w/w, formaldehyde, Fisher, Fair Lawn, NJ) were diluted in saline. Pentobarbital was obtained from Abbott Laboratories (North Chicago, IL), and halothane was supplied by Halocarbon Laboratories (River Edge, NJ).

Results

Thermal Paw Withdrawal Latency: Remifentanil

As illustrated in figure 1A, although a 30-\(\mu g/kg\) i.v. bolus of remifentanil did not change thermal paw withdrawal latency (neither did a 3-nor a 10-\(\mu g/kg\) dose; data not shown), a 90-\(\mu g/kg\) dose significantly increased thermal paw withdrawal latency. The effective duration of antinociception was shorter than phase 1 of the formalin test, so we followed the bolus injection, after a 90-sec pause, with a continuous infusion of remifentanil (45 \(\mu g/kg/hr\)) for an additional 4 min. This “0–5 min” protocol lengthened the duration of antinociception to 7 to 9 min (fig. 1A). Remifentanil also decreased respiratory rate and produced muscle rigidity and behavioral hypoactivity; these effects resolved within 1 min of the end of the infusion. Because signs of acute opioid withdrawal, including hyperalgesia, can be elicited after a single injection of morphine (Bederson et al., 1990; Kaplan and Fields, 1991), we also evaluated thermal paw withdrawal latency after the antinociception had subsided (fig. 1A). Latencies did not significantly change over time (remifentanil 90 \(\mu g/kg + 4\)-min infusion vs. saline; \(P > .05\)), which indicated that termination of remifentanil did not result in hyperalgesia.

MAP and HR: Remifentanil

Because we used formalin-evoked cardiovascular responses as a measure of nociception, it was important to determine the effects of remifentanil on MAP and HR in the absence of noxious stimulation. Table 1 depicts base-line MAP and HR, and figures 1B and 1C illustrate that remifentanil (90 \(\mu g/kg\) plus a 45-\(\mu g/kg/min\) infusion for 4 min, see above) maximally decreased MAP \((-16 \pm 8\) mm Hg) and HR \((-184 \pm 56\) bpm) after 30 and 180 sec, respectively. When the remifentanil infusion was stopped, both MAP and HR rapidly returned to predrug levels, which indicated that prolonged changes did not occur.
TABLE 1
Base-line* MAP and HR before saline or remifentanil

<table>
<thead>
<tr>
<th>Intraplantar Injection</th>
<th>Treatment (phase 1)</th>
<th>MAP (mm Hg)</th>
<th>HR (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Remifentanil i.v.</td>
<td>112 ± 3</td>
<td>417 ± 17</td>
</tr>
<tr>
<td>Formalin</td>
<td>Saline i.v.</td>
<td>107 ± 3</td>
<td>419 ± 13</td>
</tr>
<tr>
<td>Formalin</td>
<td>Remifentanil i.v.</td>
<td>108 ± 3</td>
<td>384 ± 6</td>
</tr>
<tr>
<td>Formalin</td>
<td>None</td>
<td>102 ± 3</td>
<td>400 ± 13</td>
</tr>
<tr>
<td>Saline</td>
<td>Halothane, 2.1%</td>
<td>59 ± 5</td>
<td>370 ± 11</td>
</tr>
<tr>
<td>Formalin</td>
<td>Halothane, 2.1%</td>
<td>58 ± 4</td>
<td>359 ± 11</td>
</tr>
</tbody>
</table>

* Base-line values represent group mean ± S.E. of the average of five measurements recorded just before the formalin injection. Remifentanil dose = 90 μg/kg bolus followed 90 sec later with a 4-min, 45-μg/kg/min infusion. Formalin concentration = 5% in 50 μl.

Formalin-Evoked Responses: Remifentanil

Five-minute infusions. Having established a dosing regimen for remifentanil that blocked nociceptive processing for a brief and reproducible period, we next evaluated the contribution of opioid-sensitive mechanisms during phase 1 to flinching and cardiovascular responses during phase 2. Table 1 depicts MAP and HR of the groups during the base-line period recorded just before formalin injection. Figure 2 illustrates that hindpaw formalin produced biphasic flinching and cardiovascular responses in saline controls, as observed previously (Taylor et al., 1995a). In the presence of remifentanil (0–5 min), however, we also observed transient muscle rigidity, behavioral inactivity and decreases in MAP and HR similar to those observed in the paw withdrawal experiments described above. Compared to saline, remifentanil did not change the magnitude of flinching [Group: F(1,13) = 0.51, P > .05] or pressor [Group: F(1,13) = 0.51, P > .05] responses during phase 2. Remifentanil did, however, produce a rightward shift in the MAP response during phase 2, such that the beginning of phase 2 started later and the end of the response occurred later (fig. 2B); this was indicated by a significant interaction [F(64,382) = 7.0, P < .001, Group × Time]. Also, as illustrated in figure 2C, remifentanil during phase 1 increased tachycardia responses during phase 2 [Group: F(1,13) = 9.9, P < .01], particularly after later time-points 40 to 85 (P < .005).

Fifteen- and thirty-minute infusions. Although this effect was not significant, the 0 to 15-min remifentanil administration protocol appeared to delay flinching responses during phase 2. To determine whether longer infusions would produce greater behavioral changes, we evaluated formalin-evoked flinching behavior when remifentanil was delivered from 0 to 15 or 0 to 30 min after formalin injection. Again, the effects of remifentanil on respiratory rate and behavior completely resolved within 60 sec of the end of the infusion. Figure 3A illustrates that 0 to 15-min remifentanil infusions significantly delayed the onset and termination of flinching behavior during phase 2, as indicated by a Group × Time interaction [F(14,308) = 10.5, P < .001] but did not reduce phase 2 magnitude [Group: F(1,22) = 0.033, P > .05]. Figure 3B shows that 0 to 30-min remifentanil infusions also significantly delayed the onset and termination of flinching behavior during phase 2, as indicated by a Group × Time interaction [F(14,308) = 15.2, P < .001] but only slightly reduced phase 2 magnitude [Group: F(1,22) = 4.5, P < .05].

To determine whether opioid inhibition during time-points after phase 1 would also delay formalin-evoked phase 2 responses, we next administered remifentanil during the early part of phase 2. In contrast to the delay produced by remifentanil infusion during phase 1, 15 to 30-min (fig. 3C) and 15 to 45-min (fig. 3D) infusions did not change the temporal profile of phase 2 (Group × Time: P > .05) but did decrease phase 2 magnitude [Group: F(1,22) = 5.4, P < .05; F(1,22) = 24.6, P < .001, respectively].

Formalin-Evoked Responses: Remifentanil and Naloxone Methiodide

To evaluate the hypothesis that interactions between remifentanil and peripheral opioid receptors influence cellular processes (for example, inhibition of inflammation) that then delay phase 2, we repeated the 0 to 15-min remifentanil experiment in the presence of naloxone methiodide. As illustrated in figure 4A, naloxone methiodide did not reverse the inhibition of flinching behavior produced by remifentanil during phase 1, which indicated that significant amounts of this drug did not diffuse into the CNS. Naloxone methiodide did, however, reverse the remifentanil-induced delay of phase 2 (remifentanil group vs. remifentanil and naloxone methiodide group: [F(1,13) = 70.1, P < .001; saline plus naloxone methiodide group vs. remifentanil and naloxone
methiodide group: $F(1,13) = 1.15, P > .05)$. By itself, naloxone methiodide did not change the magnitude or temporal profile of flinching behavior (fig. 4B).

Formalin-Evoked Responses: Halothane Anesthesia

Like opioid analgesics, inhalation anesthetics may also alter sensitization mechanisms in the formalin test (Abram and Yaksh, 1993; O’Connor and Abram, 1994; O’Connor and Abram, 1995). It was therefore of interest to evaluate the effect of halothane anesthesia during phase 1 on the magnitude and duration of phase 2. Figure 5 illustrates that halothane (2.1%) blocked phase 1 responses, as previously reported (Taylor et al., 1995a). Furthermore, comparison of formalin-injected animals anesthetized with halothane during phase 1 with unanesthetized controls revealed no significant difference in the magnitude of MAP and HR during time-points 15 to 45. At later time-points, however, halothane-treated animals exhibited greater MAP and HR, which indicated that phase 1 halothane extended the time to termination of phase 2 cardiovascular responses.

Table 1 shows that 2.1% halothane significantly decreased MAP and HR. To determine the magnitude of rebound increases in MAP and HR in the absence of noxious stimulation, we evaluated MAP and HR after the hindpaw injection of saline. We observed rebound increases in MAP and HR immediately after cessation of halothane (timepoints 15–30). Although formalin-treated animals exhibited similar increases over this time period, increases in the saline-treated group were over within 35 min. Therefore, rebound changes in MAP and HR did not contribute to the later period (35–85 min) of formalin-evoked cardiovascular responses during phase 2.

Discussion

Because opioid antinociception with remifentanil during phase 1 did not reduce the magnitude of phase 2 flinching and cardiovascular responses, we conclude that opioid-sensitive central sensitization mechanisms during phase 1 do not influence the magnitude of phase 2 nociceptive responses. Other studies using opioids, volatile anesthetics and local anesthetics during phase 1 of the formalin test support this conclusion. First, the i.v. injection of alfentanil just before
formalindid not decrease flinching behavior during the latter part of phase 2 (i.e., after opioid antinociception had subsided) (Gilron and Coderre, 1995), and i.v. opioid analgesia restricted to phase 1 with naloxone did not decrease flinching during phase 2 (Abram and Olson, 1994). Second, the administration of different volatile anesthetics during phase 1 did not significantly inhibit flinching during phase 2 (Abram and Yaksh, 1993; Goto et al., 1994; O'Connor and Abram, 1995).

Third, local lidocaine blockade of peripheral afferent activity during Phase 1 did not reduce the magnitude of spinal cord neuronal activity and behavioral responses during phase 2 (Haley et al., 1990; Dallel et al., 1995). In contrast to our findings, Dickenson and Sullivan (1987) and Abram and colleagues (Abram and Yaksh, 1993; Goto et al., 1994; O'Connor and Abram, 1995). Third, local lidocaine blockade of peripheral afferent activity during Phase 1 did not reduce the magnitude of spinal cord neuronal activity and behavioral responses during phase 2 (Haley et al., 1990; Dallel et al., 1995).

In contrast to our findings, Dickenson and Sullivan (1987) and Abram and colleagues (Abram and Yaksh, 1993; Goto et al., 1994; O'Connor and Abram, 1995) found that antinociception with intrathecal morphine (restricted to phase 1 with intrathecal lidocaine) greatly decreased flinching behavior during phase 2. Differences in the route of administration (i.v. vs. intrathecal) might explain this discrepancy; however, preliminary data from our laboratory indicate that intrathecal remifentanil does not reduce the magnitude of phase 2 flinching (unpublished observations). Taylor et al. submitted). Alternatively, the decreases in phase 2 observed in the latter study may be due to potential confounding factors in the intrathecal experiments: 1) residual effects of halothane administered during the formalin injection, 2) analgesic actions of naloxone, which have been demonstrated both clinically (Levine et al., 1979) and in experimental animal models of inflammatory pain (Kayser and Guilbaud, 1981; Kayser et al., 1988), including the formalin test (Vaccarino et al., 1988) and 3) termination of behavioral observation at 60 min after formalin injection, which precluded a complete analysis of shifts in the duration of phase 2. We do not believe that a reduction in phase 2 in our studies was masked by a hyperalgesia secondary to acute opioid withdrawal (Bederson et al., 1990; Kaplan and Fields, 1991), because termination of remifentanil infusion was not associated with a significant reduction in thermal paw withdrawal latency or with a persistent increase in MAP and HR.

We recently found that a quaternary lidocaine derivative (QX-314), injected 10 min after formalin into the formalin-treated hindpaw (but not the contralateral paw), completely inhibited both flinching and cardiovascular responses during phase 2 (Taylor et al., 1995a). In complementary studies, QX-314 reduced lumbar dorsal horn c-fos expression by about 50% 2 hr after formalin injection (Taylor et al., 1995b). Taken together with the fact that hindpaw formalin excites single primary afferents of low conduction velocity (C-fibers) of the sural or saphenous nerves in the barbiturate-anesthetized rat for at least 55 min (McCall et al., 1996; Puig and Sorkin, 1996), this suggests that ongoing peripheral nerve activity, rather than central sensitization, predominantly drives nociceptive responses during phase 2 in the formalin test. This ongoing activity is probably driven by multiple factors, in-
cluding: 1) direct stimulation of peripheral afferent terminals by formalin and 2) formalin-evoked neurogenic and non-neurogenic release of inflammatory mediators and other chemicals that produce peripheral sensitization.

Although remifentanil infusions during the first part of the formalin test did not change the magnitude of the flinching response during phase 2, they did produce a rightward shift, such that the beginning of flinching during phase 2 started later and the end of the response occurred later. This delay was reversed by systemic infusion of naloxone methiodide, a quaternary opiate antagonist that should not cross the blood-brain barrier to a large extent. Oluyomi et al. (1992) reported that morphine, but not quaternary morphine, reduced phase 1 pain behavior; this indicates that opioid antinociception during phase 1 is probably mediated by central opioid receptors. Because it is likely that remifentanil also reduced phase 1 via actions in the CNS, the fact that naloxone methiodide was without effect on the antinociception produced by remifentanil during phase 1 is consistent with the hypothesis that this drug reversed the remifentanil-induced delay of phase 2 by blocking peripheral opioid receptors. In summary, we hypothesize that remifentanil interacted with peripheral opioid receptors during the initial afferent barrage of the formalin test to influence the temporal profile of persistent pain.

Remifentanil is susceptible to hydrolysis by nonspecific esterases in the blood and tissue, resulting in a very rapid degradation. Using a remifentanil infusion protocol almost identical to ours (15 μg/kg/min remifentanil for 21 min), Haidar et al. (1996) recently evaluated plasma levels in rats with gas chromatography-mass spectrometry and reported that the equilibration half-life between blood and the effect compartment was only 1.7 min. These results agree with antinociception studies in the thermal tail-flick test (Feldman et al., 1991) and the thermal paw withdrawal test (current study), which showed that the duration of antinociceptive actions of high doses of remifentanil was less than 10 min. We also found that after the end of the 15 to 30- and the 15 to 45-min infusions of remifentanil (i.e., infusions began after phase 1 had subsided), flinching during phase 2 recovered to normal magnitude within 15 min. This result further indicates a short duration of action relative to the longer (30-min) effect on the temporal profile of phase 2. In humans, the terminal elimination half-life of remifentanil is 10 to 48 min (Egan et al., 1993; Glass et al., 1993; Westmoreland et al., 1993; Glass, 1995), even shorter than that of alfentanil, another short-acting opioid with an elimination half-life of 1 to 2 hr (Bower and Hull, 1982; Glass et al., 1993; Lemmens, 1995). Also, unlike other lipophilic opioids such as fentanyl and sufentanil, remifentanil does not accumulate in peripheral tissues. For example, clinical studies demonstrate that cessation of remifentanil infusion is associated with a rapid emergence of spontaneous ventilation and postoperative pain (Dershwitz et al., 1995) and with a rapid drop in remifentanil levels (Egan et al., 1993; Westmoreland et al., 1993). Indeed, the time for remifentanil concentrations to decrease by 80% remains below 15 min for infusions lasting any duration (Glass et al., 1993). Also, Feldman et al., (1991) found that the duration of action of remifentanil in the rat does not change after prolonged infusion (2 μg/min for 60 min) or after administration of multiple bolus injections (10 μg/kg × 10 i.v. injections). Because these latter studies used lower doses than the present study, however, we cannot rule out the possibility that residual concentrations of remifentanil exerted a peripheral effect on inflamed tissue that extended into phase 2. Still, the half-life of remifentanil is very short, so it is unlikely that opioid receptor interactions after the termination of remifentanil would produce the prolonged delay in the onset of phase 2 (and certainly not the delay in the offset of phase 2). Rather, we suggest that this delay results from the opioid actions of remifentanil during its infusion over phase 1.

The present studies show that inhibition of nociceptive processing with the administration of halothane during phase 1 also delayed the offset of cardiovascular responses during phase 2. Abram and colleagues (Abram and Yaksh, 1993; O’Connor and Abram, 1995) reported that the delivery of isoflurane, enflurane, desflurane or halothane restricted to phase 1 delayed the onset of flinching behavior during phase 2; however, as we noted in the Introduction, they did not evaluate the time to termination of phase 2 and thus concluded that the magnitude of phase 2 had decreased. In another animal model, Seltzer et al. (1991) reported that the onset of autotomy after peripheral nerve section is delayed by pre-emptive local anesthesia of the nerve. Thus, early nociceptive events produced by acute tissue or nerve injury may influence the temporal profile of tonic nociceptive responses in different models of persistent pain.

What mechanisms might underlie the delay in the onset and termination of phase 2 after interactions between remifentanil and opioid receptors? As discussed above, peripheral opioid receptors are probably involved. Because opioids have anti-inflammatory actions (Joris et al., 1990; Barber and Gottschlich, 1992), including inhibition of the calcium-dependent release of proinflammatory peptides from peripheral nerve endings (Yaksh, 1998), and because interactions of opioids with peripheral opioid receptors can reduce the edema evoked by intraplantar formalin during both phase 1 and phase 2 (Wheeler-Aceto and Cowan, 1991; Hong and Abbott, 1995), we hypothesize that phase 2 was delayed because remifentanil retarded the formalin-evoked synthesis and/or release of proinflammatory compounds from peripheral tissues during phase 1. It is consistent with this hypothesis that when we administered remifentanil after phase 1, i.e., after the release of inflammatory mediators had presumably been initiated, we found no change in the time course of phase 2. Although peripheral analgesia with a relatively hydrophilic opiate such as morphine is difficult to demonstrate in normal (uninflamed) tissue, we suggest that remifentanil interacted with opioid receptors during phase 1, i.e., before the period of maximal inflammation, for the following reasons: 1) It is likely that formalin disrupted the perineurium. Because perineurial permeability is correlated with peripheral opioid analgesia (Antonijevic et al., 1995), such a disruption could facilitate access of remifentanil to neuronal opioid receptors. 2) Antonijevic et al. (1995) also found that peripheral administration of fentanyl, a highly lipophilic opioid agonist similar in structure to remifentanil, produced strong analgesic effects even in noninflamed tissue. 3) Formalin injection into the hindpaw probably produces some neurogenic inflammation during phase 1, though to a lesser extent than during and after phase 2. Indeed, formalin increased plasma extravasation in the hindpaw within 15 min (Hong and Abbott, 1995; Taylor et al., unpublished observations). We suggest that the target for the delay effect of
Remifentanil includes opiate receptors located on peripheral terminals of primary afferents (Fields et al., 1980; Young et al., 1980; Stein et al., 1990). To address these hypotheses, future studies will evaluate the effects of remifentanil on the time course of formalin-evoked inflammation.

In summary, we found that opioid-sensitive mechanisms during phase 1 influence the temporal profile, but not the magnitude, of phase 2 responses to hindpaw formalin injection. Because it is likely that inflammatory mediators excite primary afferents during phase 2, we hypothesized that remifentanil interacted with opioid receptors in peripheral tissue to delay the formalin-evoked synthesis and/or release of proinflammatory compounds and thus delayed the onset and termination of phase 2.

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References


