The μ-opioid agonist remifentanil attenuates hyperalgesia evoked by blunt and punctuated stimuli with different potency: a pharmacological evaluation of the freeze lesion in humans

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Abstract

Experimental pain models inducing hyperalgesia, i.e. an increased sensitivity to noxious stimuli often present in clinical pain, are important tools for studying antinociceptive drug profiles. The correct interpretation of results obtained in these models necessitates their mechanistic understanding. This study evaluated the freeze lesion, an experimental model of hyperalgesia, in humans. Twelve healthy subjects were tested with mechanical (brush, punctuated and blunt) and electrical (5, 250, and 2000 Hz sine wave current) stimuli before and after freezing the skin, and during a computer-controlled infusion of the μ-opioid agonist remifentanil targeting five different plasma concentrations between 0 and 6 ng/ml in a two-staged, single occasion, randomized, and double blind study design. Pharmacodynamic modeling techniques were used to describe the effect of freezing and drug administration on the mechanical and electrical pain thresholds. Freezing the skin resulted in hyperalgesia to blunt and punctuated stimuli and lowered the respective pain threshold by 29 and 73%. Hyperalgesia to brushing or electrical stimuli was not detected. Remifentanil attenuated hyperalgesia to blunt stimuli about twice as potently as hyperalgesia to punctuated stimuli, as indicated by a significantly steeper linear relationship between the remifentanil plasma concentration and the increase of the pain threshold to blunt stimuli. Remifentanil attenuated electrical pain with greater potency for low frequency stimulation. The potency difference of remifentanil suggests that different neuronal mechanisms mediate hyperalgesia to blunt and punctuated stimulation. Absence of brush-evoked and electrical hyperalgesia is compatible with the view that mechanical hyperalgesia to blunt and punctuated stimulation of the freeze lesion is predominantly caused by a peripheral mechanism.

1. Introduction

Experimental pain models are important tools to study the antinociceptive profile of pharmacological compounds. Models inducing hyperalgesia, i.e. a condition resulting in an enhanced pain response to noxious stimuli, and allodynia, i.e. a pain response to non-noxious stimuli, seem particularly relevant because clinical pain is often associated with hyperalgesia and allodynia. Hyperalgesia and allodynia have been induced experimentally with chemical and electrical stimulation, and by injuring tissue with a burn or a freeze lesion. A mechanistic understanding of these experimental models is crucial for the correct interpretation of results obtained with them.

From a descriptive and mechanistic point of view hyperalgesia is a heterogeneous and just a partially understood phenomenon. The hyperalgesic zone at the site of injury is called primary, whereas the hyperalgesic zone surrounding the site of injury is called secondary. Hyperalgesia to heat stimulation is present in the primary zone and is likely to be due to sensitized peripheral nociceptors (Raja et al., 1984; Treede and Magerl, 2000). Hyperalgesia to mechanical stimulation is more complex and at least three subtypes seem to exist. Mechanical allodynia elicited with gentle brush is present in the primary and secondary zone and is most likely to be caused by the enhanced central processing of sensory input (central sensitization) from large myelinated nerve fibers (Aβ) (Price et al., 1989; Koltzenburg et al., 1992; Torebjork et al., 1992; Leem et al., 1993; Ochoa

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Similarly, mechanical hyperalgesia to punctuated stimuli (von Frey hair) is present in both zones and is predominantly mediated by small myelinated nerve fibers (Aβ, Aδ) (Schaedty et al., 1983; LaMotte et al., 1991; McAllister et al., 1995; Ziegler et al., 1999; Treede and Magerl, 2000). Central sensitization to sensory input from Aδ-fibers likely explains the response observed in the secondary zone (Ziegler et al., 1999). However, the mechanism underlying the response observed in the primary zone is less certain. Recent evidence supports an important role for sensitized peripheral mechano-nociceptors (Cooper et al., 1991; Andrew and Greenspan, 1999; Schmelz et al., 2000; Ringkamp et al., 2001). Finally, mechanical hyperalgesia evoked by blunt pressure has been documented within the primary zone and may be due to sensitized C-nociceptors (C-fibers) (Cline et al., 1989; Culp et al., 1989; Koltzenburg et al., 1992; Kilo et al., 1994).

The existing mechanistic concept concerning mechanical hyperalgesia results from neurophysiological studies. The primary aim of this investigation was to examine and expand this concept with a pharmacological study in humans. The primary aim of this investigation was to examine and expand this concept with a pharmacological study in humans. Using the freeze lesion as an experimental model inducing stable hyperalgesia, the hypothesis that the μ-opioid receptor agonist remifentanil attenuates the hyperalgesic response evoked by brush, punctuated, and blunt stimulation with increasing potency was tested. Verification of this hypothesis would provide further evidence that hyperalgesia elicited by brush, punctuated, and blunt stimulation is predominantly mediated by Aβ, Aδ, and C-fibers because opioids are known to attenuate sensory input from these fiber classes with increasing potency (Le Bars et al., 1976; Cooper et al., 1986; Wang et al., 1996). A secondary aim of this investigation was to test the hypothesis that electrical hyperalgesia does not exist in the primary zone of mechanical hyperalgesia. Verification of this hypothesis would provide further support for a predominant role of a peripheral mechanism underlying mechanical hyperalgesia to punctuated stimulation within the primary zone. Electrical hyperalgesia has been documented in models causing hyperalgesia by central sensitization, but would not be expected in models causing hyperalgesia by sensitizing specific peripheral nociceptors (Price et al., 1989; Simone et al., 1991; Torebjork et al., 1992; Arendt-Nielsen et al., 1996).

2. Methods

This report summarizes experimental pain data obtained in human volunteers participating in a two-staged study protocol. First, the mechanical and electrical pain thresholds were determined in skin before and after induction of experimental hyperalgesia. Second, the potency of the μ-opioid receptor agonist remifentanil for reversing the hyperalgesic response to distinct mechanical and electrical stimuli was determined.

2.1. Clinical protocol

The study was approved by the Institutional Review Board of Stanford University, and 12 healthy subjects (Table 1) were enrolled after having given written informed consent. Prior to participation in the study, subjects fasted for at least 6 h and abstained from over-the-counter medication for a minimum of 48 h. Intake of prescription drugs during the 14 days preceding the study was prohibited except for the use of oral contraceptives.

A double blind, randomized, single occasion study design was used. Each subject was studied on 2 consecutive days undergoing a total of six pain test cycles. During a cycle, three different mechanical and three different electrical pain tests were performed. On day 1, a subject underwent the first test cycle in a marked, non-inflamed skin area on each thigh.
After completion of the first test cycle the two skin areas were briefly frozen to induce inflammation and hyperalgesia. Subjects returned to the study center on the next day (half of the subjects at 8 a.m. and 13 p.m., respectively). Three test cycles were performed before and two cycles after starting a target-controlled infusion of the \(\mu\)-opioid agonist remifentanil. Twelve subjects were randomly assigned to receive one of the following six target sequences: 0 and 3.6, 1.2 and 4.8, 2.4 and 6.0, 3.6 and 0, 4.8 and 1.2, 6.0 and 2.4 ng/ml. Each sequence was assigned to one man and one woman. As an example the target concentration (dotted line), and the resulting plasma (dashed line) and effect site concentration (solid line) are depicted versus time. During drug infusion the mechanical and electrical pain thresholds were determined after achieving steady state plasma and effect site concentrations. The time course of the plasma and the effect site concentration was simulated with the software Stanpump (see text for reference).

2.2. Remifentanil infusion

The effects of remifentanil were studied at six target plasma concentrations including zero, i.e. at 0, 1.2, 2.4, 3.6, 4.8, and 6.0 ng/ml. Subjects were not studied on a separate occasion with saline placebo because one of the six target concentrations was equal to zero. The target concentrations were chosen to study the effect of remifentanil across a wide concentration-range without producing severe respiratory depression or sedation. Two different target concentrations were studied per subject (Table 1). Each of the six target plasma concentrations was randomly assigned as the first target plasma concentration to be achieved in one man and one woman. The second target concentration was contingent on the first one, i.e. was 3.6 ng/ml higher than the first one if the first target concentration ranged between 0 and 2.4 ng/ml, and was 3.6 ng/ml lower if the first target concentration ranged between 3.6 and 6.0 ng/ml. This paradigm was chosen to allow equilibration of the second target concentration between plasma and the effect site within 15 min. Greater differences between the first and second target concentrations would have required longer equilibration times.

On arrival at the study center a catheter was placed into a vein of the left arm and recording of vital signs was started (electrocardiogram (ECG), arterial blood pressure, hemoglobin oxygen saturation, and respiratory rate). Remifentanil (Glaxo Welcome, Research Triangle Park, NC) was administered intravenously by a computer-controlled infusion pump (Harvard Pump 22, Harvard Apparatus Inc.,

![Image](53x406 to 535x727)

Fig. 1. The study flow is depicted schematically along the time axis. Thresholds for mechanical (brush, punctuated, and blunt stimuli) and electrical (sine wave current at 5, 250, and 2000 Hz) pain were determined before setting a freeze lesion at each thigh (inset graph). Twenty-four hours after setting the freeze lesion mechanical and electrical pain thresholds were determined three times before starting a target controlled infusion of the \(\mu\)-opioid agonist remifentanil. Twelve subjects were randomly assigned to receive one of the following six target sequences: 0 and 3.6, 1.2 and 4.8, 2.4 and 6.0, 3.6 and 0, 4.8 and 1.2, 6.0 and 2.4 ng/ml. Each sequence was assigned to one man and one woman. As an example the target concentration (dotted line), and the resulting plasma (dashed line) and effect site concentration (solid line) are depicted versus time. During drug infusion the mechanical and electrical pain thresholds were determined after achieving steady state plasma and effect site concentrations. The time course of the plasma and the effect site concentration was simulated with the software Stanpump (see text for reference).
South Natick, MA) to quickly achieve and maintain a target plasma concentration (Shafer et al., 1990). STANPUMP was the software used to drive the infusion pump (copyright by Steven L. Shafer, Palo Alto Department of Veterans Affairs Medical Center, Palo Alto, CA; software available on the World Wide Web at http://pkpd.icon.palo-alto.med.va.gov). The pharmacokinetic parameters used with STANPUMP have previously been validated (Drover and Lemmens, 1998).

Subjects and investigators knew that remifentanil (including a zero dose) was administered during the last two test cycles. However, they were blinded with respect to the actual target plasma concentration. Subjects and the blinded investigator had no sight of the arm with the IV-access and of the remotely placed infusion pump to prevent giving them clues about the infusion algorithm. This was important because the infusion rate varied according to the target plasma concentration and was zero if the target plasma concentration was zero.

2.3. Induction of cutaneous inflammation (freeze lesion)

Cutaneous inflammation was induced by briefly freezing skin at the anterior part of each thigh 22–24 h prior to testing as described by Kilo et al. (1994). Briefly, a copper cylinder weighing 290 g and of 15 mm in diameter was cooled to −28°C and placed perpendicularly onto the skin for 10 s. No additional pressure was exerted on the copper cylinder during the procedure. For better thermal contact, a filter paper soaked with saline was placed between skin and the copper cylinder. Induction of the freeze lesion was slightly painful, evoking a perception of electrical prickle, and rarely burning, as reported previously (Kilo et al., 1994). Any spontaneous pain subsided within 2 h. However, the skin lesion remained slightly reddened and sensitive to mechanical stimulation for a few days.

The freeze lesion was evaluated as a model of cutaneous inflammation for two reasons. First, the lesion is very well standardized, i.e. the temperature, the pressure and the exposure time used for induction of the lesion can be tightly controlled. Second, the lesion provides stable test conditions 1 day after its induction because the lesion develops and ceases over a period of days. A well-standardized lesion offering stable test conditions is an important consideration when choosing an experimental inflammatory model for pharmacological studies.

2.4. Experimental pain testing

Different noxious mechanical and electrical stimuli were used for preferential stimulation of different nerve fibers. Mechanical testing included administration of brushing, punctuated, and blunt stimuli to the inflamed skin area. Neurophysiological and psychophysical studies in humans suggest that pain evoked by applying brushing, punctuated, and blunt stimuli to inflamed and hyperalgesic skin is transmitted via large myelinated nerve fibers (Aβ), small myelinated nerve fibers (Aδ), and unmyelinated C-fibers, respectively (Culp et al., 1989; Koltzenburg et al., 1992; Torebjork et al., 1992; Leem et al., 1993; Kilo et al., 1994; Ziegler et al., 1999).

Electrical testing was performed with a constant current device (Neurometer, Neurotron Inc., Baltimore, MD) designed to deliver bi-phasic sine wave stimuli at 5, 250, and 2000 Hz. The three stimulation frequencies are thought to evoke sensations caused by a differential neuronal input from small and large fibers. Increasing the stimulation frequency preferentially reduces the contribution of small fiber input to an overall sensation because the maximum firing frequency is lowest for these fibers (Torebjork and Hallin, 1974). Nerve conduction studies and comparative quantitative sensory tests in patients with a sensory deficit, as well as pharmacological studies in animals and humans provide indirect evidence that C-fiber input is predominant for sensations evoked with 5 Hz and Aβ-fiber input is predominant for sensations evoked with 2000 Hz (Masson et al., 1989; Pitei et al., 1994; Veves et al., 1994; Liu et al., 1995; Wallace et al., 1996; Kiso et al., 2001) Some pharmacological studies suggest that Aδ-fiber input may be predominant for sensations evoked with 250 Hz (Liu et al., 1995; Wallace et al., 1996; Kiso et al., 2001). However, this is less certain. In analogy to the mechanical testing, the three electrical stimulation frequencies were used to test whether electrical hyperalgesia could be documented for neuronal input predominantly arising from small and large afferent fibers (5 Hz for C-fibers and 2000 Hz for Aβ-fibers), and whether the μ-opioid agonist, remifentanil, attenuated pain caused by the three different electrical frequencies with differential potency. All testing was done within the area of skin directly subjected to thermal injury.

2.4.1. Stroking stimulus

Four different brushes (Series 235, Winsor and Newton, England, UK) were used in randomized order for gently brushing the freeze lesion. Starting 2 cm outside of the lesion each brush was moved three times across the lesion at a rate of about 2 cm/s, thereby exerting a load of approximately 1.5, 3.5, 5, and 11.5 g. The load at the tip of a brush was determined while moving the brush by hand at 2 cm/s and at a 60° angle over a scale. The weight of each brush determined the load that the brush exerted at its tip. Care was taken not to exert any additional load when holding the brush by hand (two fingers). The interval between strokes was about 5 s. After each stroke the subject had to indicate if pain had been evoked. Subjects kept their eyes closed during the testing.

2.4.2. Punctuated stimulus

Von Frey hairs of different strength (1, 1.3, 2.2, 4.7, 6.2, 7, 9, 14, 20, 45, 60, 90, and 140 g; Research Designs Inc., Houston, TX) were placed perpendicularly onto the freeze lesion and bent slightly to apply punctuated pressure. Starting with the thinnest hair, consecutively thicker hairs were
used until a subject reported pain for the first time. Subsequently, the same or the next thinner hair was used if pain had been reported for the preceding stimulus, or the same or the next thicker hair was used if no pain had been reported for the preceding stimulus. Random choice determined as to which of the two hairs was used. The procedure was repeated until a subject had reported four times that a non-painful stimulation had changed to a painful stimulation and three times that a painful stimulation had changed to a non-painful stimulation, respectively. This way seven data points were obtained reflecting the strengths of the von Frey hair that caused a change from a non-painful to a painful perception and vice versa. The mechanical pain threshold to stimulation with von Frey hairs was defined as the geometric mean of these seven data points. Subjects kept their eyes closed during the testing.

2.4.3. Blunt pressure stimulus

A pressure algometer with a circular and flat probe of 1 cm diameter (Commander Algometer, J Tech Medical Industries, Midvale, Utah; maximum output = 111.6 N/cm²) was placed perpendicularly onto the freeze lesion. The pressure was increased at a rate of approximately 9 N/cm²/s until the subject indicated pain. The increase in pressure was controlled manually by the investigator and stopped once the subject reported pain. The maximum pressure applied was recorded automatically. The procedure was repeated four times at intervals of 30 s. The mechanical pain threshold to blunt pressure was calculated as the median of the five measurements.

2.4.4. Electrical stimulation

A constant current device (Neurometer, Neurotron Inc., Baltimore, MD) with a maximum output of 20 mA delivered bi-phasic sine wave pulses of 3 s duration at 5, 250, and 2000 Hz. The inter-stimulus interval was 15 s. An aluminum/gold electrode was attached to the surface of the freeze lesion and the electrical pain threshold was determined as previously described (Angst et al., 2000). Briefly, stimulation started at a current known not to inflect pain. Current was subsequently increased using an ascending staircase design until pain was reported for the first time. Current was then up and down regulated based on a subject’s report of a non-painful or painful perception until the difference between the maximum current not evoking pain and the minimum current evoking pain was not greater than 10%. The average of those two values was defined as the electrical pain threshold.

2.5. Side effects

All side effects spontaneously reported by the subjects were recorded. During drug infusion subjects were requested to indicate if they felt sedated, nauseated, itchy, or euphoric on a categorical scale (none, mild, moderate, and severe).

2.6. Data analysis

A pharmacodynamic modeling approach was used to describe the mechanical (brushing, punctuated pressure, and blunt pressure) and electrical (5, 250, and 2000 Hz) pain thresholds over the course of the experiment. The pain threshold was expressed as the percentage change relative to the threshold determined before inducing the freeze lesion. The pharmacodynamic model used for this analysis was based on a modeling approach previously used for the description of human pain data (Luginbuhl et al., 2001). The model described the change of the pain threshold as the sum of (1) a change due to freezing of the skin, (2) a change due to a drug-independent shift of the threshold over time, and (3) a change due to administration of remifentanil. The complete model described each of the three components by a separate mathematical term:

\[
\text{%change in threshold} = \text{freeze effect} + \text{slope}_{\text{time}} \frac{\%}{h} \text{time (h)} + \text{slope}_{\text{remi}} \frac{\%}{\text{ng/ml}} \text{concentration}_{\text{remi}} \gamma (\text{ng/ml})
\]

(1)

where % denotes the percentage change of the threshold relative to the threshold measured before induction of the freeze lesion (absolute thresholds were measured in grams for blunt stimuli, in Pascal for punctuated stimuli, and in milliampere for electrical stimuli).

The model was based on the following three stipulations: (1) A change of the pain threshold due to freezing of the skin is quantified by the parameter ‘freeze effect’. (2) An effect of time on the pain threshold is described by a linear model (% change in threshold = slope_{time} \times time). A linear model was chosen because, from a statistical point of view, it is the most sensitive model to detect a change of the pain threshold over time. (3) The effect of remifentanil on the pain threshold is described by a power model (% change in threshold = \text{slope}_{\text{remi}} \times \text{concentration}_{\text{remi}} \gamma). A power model (linear or non-linear) was chosen because the data did not support a sigmoidal model, i.e. no ceiling effect was observed for the pain threshold at the highest plasma concentrations.

Gender and the daytime of the experiment were built-in covariates of the pharmacodynamic model because they might have affected the pain threshold. As an example, the effect of gender on the relationship between the remifentanil plasma concentration and the pain threshold was examined by introducing the covariate \theta into the relevant portion of pharmacodynamic model (threshold = \text{slope}_{\text{remi}} \times \theta \times \text{concentration}_{\text{remi}} \gamma). For one gender \theta was fixed at a value of 1, whereas for the other gender \theta was estimated during the fitting procedure. A significant gender difference was present if \theta significantly improved the fit (see subsequently) and the estimated \theta differed significantly from a
Fig. 2. Individual thresholds for mechanically (punctuated and blunt stimulation) and electrically (5, 250, 2000 Hz) induced pain are shown. Squares (■) indicate the pain threshold before induction of the freeze lesion. Circles (○) indicate the pain threshold determined after inducing the freeze lesion, but before administering the μ-opioid agonist remifentanil. Filled triangles (▲) indicate thresholds determined during the drug infusion. Solid lines depict the course of the mechanical and electrical pain thresholds as predicted by the corresponding pharmacodynamic model. The target concentration of remifentanil is noted below each individual data point obtained during drug infusion (‘first Remi’ and ‘second Remi’, for first and second remifentanil target concentration). Freezing the skin resulted in a drop of the mechanical pain threshold to punctuated and blunt stimulation (top two graphs). However, the electrical pain threshold after freezing the skin was slightly elevated (bottom three graphs). Infusion of the μ-opioid agonist remifentanil resulted in a dose-dependent antihyperalgesic (mechanical stimulation) and analgesic (electrical stimulation) effect.
value of 1 (95% confidence interval not including a value of 1).

Data were fitted with non-linear mixed effects modeling techniques. The software NONMEM was used for estimation and validation of each model parameter (NONMEM V version 1.1, NONMEM Project Group, UCSF, CA). The procedure is described in detail in the NONMEM user’s guide (Beal and Sheiner, 1979) and reveals whether a model parameter has statistical significance by applying goodness-of-fit criteria. NONMEM uses the negative product of two times the log-likelihood (−2LL) to quantify the goodness of fit (likelihood ratio test based on a Chi squared distribution). If adding a parameter to the pharmacodynamic model decreases the value of −2LL by 3.84, a statistically significant improvement of the fit at an α-level of 0.05 has been proven and the parameter becomes part of the model. Parameters that did not prove statistically significant were eliminated from the model. Visual inspection of the fit ascertained that the final model adequately described the data.

Two procedures were used to compare the potencies of remifentanil for attenuating hyperalgesia to blunt and punctuated stimulation. First, the individual slopes of the linear relationship describing the effect of remifentanil on blunt and punctuated hyperalgesia were compared with a paired t-test. Second, the individual remifentanil plasma concentrations necessary to reverse mechanical hyperalgesia to blunt and punctuated stimulation were calculated (brush did not evoke pain; electrical hyperalgesia was not observed). A t-test was used to test whether the potencies of remifentanil (i.e. the plasma concentrations) for reversing hyperalgesia to blunt and punctuated stimuli were significantly different. A valid comparison of the two potencies had to take into account that hyperalgesia was more pronounced for punctuated than for blunt stimulation (reduction in pain threshold by 73 and 29%, respectively). Failure to account for this difference would have overestimated the potency for reversing the less pronounced form of hyperalgesia, i.e. hyperalgesia to blunt stimulation. Individual differences were compensated for by dividing the plasma concentration necessary for reversing hyperalgesia to punctuated stimulation by the ratio between the magnitude of hyperalgesia to punctuated and blunt stimulation before drug administration. This linear correction was appropriate because the relationship between the remifentanil plasma concentration and the anti-hyperalgesic effect to punctuated and blunt stimulation was linear.

To compare the potency of remifentanil for attenuating electrical pain, the individual slopes of the linear relationship describing the effect of remifentanil on pain elicited with 5, 250, and 2000 Hz stimulation were compared with a one-way repeated measures analysis of variance and post hoc t-test multiple comparison with Bonferroni’s correction. A one-way repeated measures analysis of variance was used to test whether the pain threshold measured three times after freezing the skin but before administering drug changed over time. Spearman rank order correlation was used to test for a significant association between the rating of side effects (none, mild, moderate, and severe) and the remifentanil plasma concentration. All non-modeling statistics were performed with SPSS (version 10.1.4 for Windows™, SPSS Inc., Chicago, IL, USA). The α-level was set at 0.05. All results are expressed as the mean and the standard deviation if not stated otherwise.

### 3. Results

All 12 subjects completed the study according to the protocol. No adverse events were observed. Oral contraceptives were the only ongoing drug therapy during the study.

#### 3.1. Experimental pain

**3.1.1. Stroking pain**

Brushing of the freeze lesion never evoked pain. Slightly stroking the lesion with the investigator’s fingertip as an alternative procedure also failed to evoke pain (Ma and Woolf, 1997).
3.1.2. Punctuated pain

Freezing the skin resulted in significant mechanical hyperalgesia to punctuated stimulation (Fig. 2). The pain threshold dropped by 73 ± 22% (Table 2). Remifentanil attenuated mechanical hyperalgesia to blunt pressure in a linear and dose-dependent fashion (Fig. 3). The pain threshold did not shift over time and was not significantly affected by gender or daytime. The estimated remifentanil plasma concentration necessary to reverse the hyperalgesic effect in the studied population was 1.0 ng/ml. The final pharmacodynamic model was composed of the same parameters as the model derived for punctuated stimulation. All model parameters are presented in Table 2.

Remifentanil attenuated hyperalgesia to blunt stimulation about two times more potently than hyperalgesia to punctuated stimulation. This is indicated by the significantly steeper slope (about two times) that characterizes the relationship between the remifentanil plasma concentration and the reduction of pain evoked by blunt stimulation (Fig. 3). Further evidence is provided by the fact that a plasma concentration of 1.0 and 5.2 ng/ml was required to reverse hyperalgesia to blunt and punctuated stimulation (Fig. 3). Accounting for the different magnitude of hyperalgesia to blunt and punctuated stimulation after freezing the skin (see Section 2.6) this also indicates a potency difference by a factor of about 2 (P < 0.05).

3.1.4. Electrical pain

Freezing the skin did not result in electrical hyperalgesia (Fig. 2). Remifentanil alleviated electrically induced pain in a linear and dose-dependent fashion. The pain threshold did not shift over time and was not significantly affected by gender or daytime. The slopes characterizing the relationship between the remifentanil plasma concentration and the pain threshold differed significantly among the three frequencies used for electrical stimulation. The slope was steepest for 5 Hz and flattest for 2000 Hz stimulation (Table 2). However, post hoc testing only revealed a significant difference between 5 and 250 Hz stimulation (Table 2). The final model describing these effects is given by Eq. (2) and model parameters are listed in Table 2.

3.2. Side effects

Side effects were of mild to moderate severity and typical for opioid administration (itching, nausea, occasional vomiting, blurred vision, drowsiness). The occurrence and magnitude of sedation (r = 0.66) and pruritus (r = 0.85) correlated significantly with the remifentanil plasma concentration. No correlation was observed between the remifentanil plasma concentration and nausea or euphoria.

4. Discussion

In human volunteers the μ-opioid receptor agonist remifentanil attenuated the hyperalgesic response to blunt
stimuli with significantly greater potency than the hyperalgesic response to punctuated stimuli. These results add pharmacological evidence to neurophysiological studies suggesting that mechanical hyperalgesia evoked with blunt and punctuated stimuli at the site of injury (primary hyperalgesia) are distinct forms of mechanical hyperalgesia and should be distinguished.

The observation that remifentanil attenuated hyperalgesia to blunt stimuli more potently than hyperalgesia to punctuated stimuli is consistent with the hypothesis that pain evoked by blunt stimulation is mainly mediated by C-nociceptors and pain evoked by punctuated stimulation is predominantly mediated by Aδ-nociceptors. A more potent attenuation of C-fiber than of Aδ-fiber mediated responses by opioids has consistently been demonstrated in experiments using techniques as different as single cell recordings and psychophysics (Le Bars et al., 1976; Cooper et al., 1986; Wang et al., 1996). From a neurophysiological perspective, studies using selective nerve fiber block techniques, micro-neurography, and single fiber recordings suggest a predominant role of C- and Aδ-nociceptors for transmitting a hyperalgesic response to blunt and punctuated stimuli, respectively (Schady et al., 1983; Cline et al., 1989; Culp et al., 1989; LaMotte et al., 1991; Koltzenburg et al., 1992; Kilo et al., 1994; McAllister et al., 1995; Ziegler et al., 1999; Treede and Magerl, 2000).

This study documents the presence of mechanical hyperalgesia, but absence of electrical hyperalgesia at the site of skin injury. The absence of electrical hyperalgesia may provide further insight into the mechanism underlying the development of primary mechanical hyperalgesia.

Studies examining the stimulation threshold of mechano-nociceptors could not document a reduction of the threshold in inflamed skin and supported the view that primary mechanical hyperalgesia was predominantly due to enhanced spinal processing or central sensitization (Treede et al., 1992). However, two studies recording from single units in the peripheral nerve provide evidence that inflammation can result in sensitization of Aδ and C mechano-nociceptors for supra-threshold stimuli while the stimulation threshold may remain unchanged (Cooper et al., 1991; Andrew and Greenspan, 1999). In addition, a special class of receptors, the mechano-insensitive receptors have been identified and they likely contribute to the development of primary mechanical hyperalgesia (Schmelz et al., 2000; Ringkamp et al., 2001). Intradermal injection of capsaicin in humans or monkeys sensitized these receptors to mechanical stimuli. Based on these findings primary mechanical hyperalgesia cannot only be explained by central sensitization, but also by the peripheral sensitization of nociceptors.

Our observation that electrical hyperalgesia was absent at the site of skin injury is compatible with a peripheral mechanism for the development of primary mechanical hyperalgesia. It seems reasonable to assume that the electrical excitability of the axon would not change significantly after peripheral sensitization of specific mechanoreceptors. However, an increased pain response to electrical and not only mechanical stimulation might be expected if mechanical hyperalgesia elicited at the site of injury was a central phenomenon, i.e. was caused by enhanced spinal processing of unaltered peripheral neuronal input. Several studies support this view. In monkeys a significantly increased response rate of spinothalamic tract neurons to electrical stimulation of the cut proximal dorsal rootlet was demonstrated after intradermal injection of capsaicin into the receptive field (Simone et al., 1991). In a human experiment direct axonal electrical stimulation of nerve fibers normally signaling tactile sensations also evoked allodynia after the injection of capsaicin in close proximity to the receptive field (Torebjork et al., 1992). In human volunteers a significantly enhanced pain response to electrical stimulation of the secondary, but not of the primary hyperalgesic skin zone was reported after exposure to capsaicin (Arendt-Nielsen et al., 1996). Finally, a decreased electrical pain threshold has been reported in patients suffering from complex regional pain syndrome, a chronic pain condition associated with facilitated central neuronal processing (Raj et al., 2001). However, the input characteristics of electrical and mechanical stimuli into the central nervous system (CNS) are rather different. The lack of electrical hyperalgesia within the freeze lesion only provides indirect evidence for a peripheral mechanism underlying the observed mechanical hyperalgesia.

High plasma concentrations of remifentanil not only reversed mechanical hyperalgesia to blunt stimulation, but also resulted in an apparent analgesic effect, i.e. it raised the pain threshold to a value greater than normal (that observed before inducing the freeze lesion). Raising the pain threshold back to a normal level (pain threshold before skin injury) could be labeled as anti-hyperalgesic drug action, while elevating the pain threshold above normal could be labeled as analgesic drug action. However, such a distinction is based on phenomenological and not on mechanistic considerations. The processing of nociceptive input is different under hyperalgesic and non-hyperalgesic conditions. This mechanistic difference remains, even if the pain threshold has been elevated above normal. Therefore, we used the term anti-hyperalgesic drug action for any drug effect measured in a hyperalgesic condition, and the term analgesic drug action for any drug effect measured in a non-hyperalgesic condition.

Brushing the freeze lesion did not elicit pain. This finding is in agreement with a previous report documenting that allodynia, i.e. pain evoked by light touch is not a consistent feature of the freeze lesion (Kilo et al., 1994). Mechanical allodynia is due to the altered central processing of mechano-receptive input from nerve fibers transmitting a non-painful, tactile sensation (Torebjork et al., 1992). The expression of mechanical allodynia depends on the continuous discharge of peripheral nociceptors (Treede and Magerl, 2000). Induction of the freeze lesion is accompanied by little spontaneous pain that has long ceased when
hyperalgesia develops within the freeze lesion (Kilo et al., 1994). Absence of spontaneous pain suggests little or no continuous discharge from nociceptors, and this may explain why mechanical allodynia is not a typical feature of the freeze lesion.

Absence of mechanical allodynia, and presence of mechanical hyperalgesia that may be explained by the peripheral sensitization of mechanoreceptors raises the question whether true secondary hyperalgesia, i.e. hyperalgesia due to central sensitization is a characteristic feature of the freeze lesion. A previous study has reported mechanical hyperalgesia to punctuated stimuli and mild but significant hyperalgesia to heat stimuli in skin adjacent to the freeze lesion (Kilo et al., 1994). The authors suggested that this form of hyperalgesia was due to central sensitization, because the skin site had not been injured directly. However, thermal hyperalgesia is not a typical finding in secondary hyperalgesia and the area of mechanical hyperalgesia surrounding the freeze lesion was relatively small (a halo with a width of about 2 cm) (Raja et al., 1984; Treede and Magerl, 2000). Hyperalgesia in the zone surrounding the freeze lesion may be a form of secondary hyperalgesia. However, an alternative mechanism should be considered. This zone may reflect an area of extended primary hyperalgesia, i.e. could be due to the local spreading of an inflammatory response during the 24-h period between freezing the skin and testing for hyperalgesia. The strict topographical distinction between primary and secondary hyperalgesia (hyperalgesia at the site of injury and hyperalgesia surrounding the site of injury) may not reflect the underlying mechanism rendering these skin sites hyperalgesic (peripheral versus central sensitization).

Increasing the electrical stimulation frequency lowered the potency of remifentanil for attenuating electrical pain. It seems reasonable to suggest that this may be due to a frequency-dependent, differential contribution of unmyelinated and myelinated nociceptive fibers to the overall perception of pain. As discussed previously, the opioid remifentanil is expected to attenuate noxious C-fiber input more potently than noxious A-fiber input (Le Bars et al., 1976; Cooper et al., 1986; Wang et al., 1996). Therefore, our results suggest that the contribution of nociceptive C-fiber input to the perception of electrical pain is most prominent for 5 Hz stimulation and least important for 2000 Hz stimulation. This observation is in agreement with the excitability characteristics of different nerve fiber classes. First, decreasing the stimulus duration (equivalent to increasing the frequency in this experiment) is expected to preferentially increase the excitation threshold of unmyelinated nerve fibers because chronaxie is greatest in these fibers. Second, the maximum firing frequency is lowest in unmyelinated fibers (Torebjork and Hallin, 1974). In other words, C-fibers become particularly difficult to excite and are the first displaying a ceiling in firing frequency as the stimulation frequency is increased.

In summary, this study presents pharmacological evidence in humans supporting the view that hyperalgesia to blunt pressure and punctuated stimuli is mediated by C- and Aδ-fibers, respectively. Distinction between these subtypes of mechanical hyperalgesia seems to be crucial for the correct interpretation of results obtained with mechanical stimulation of a hyperalgesic lesion. This study also supports the view that mechanical hyperalgesia at the site of injury is predominantly due to the peripheral sensitization of nociceptors. Finally, this study confirms that the freeze lesion is an adequate model of primary hyperalgesia, but raises the question whether secondary hyperalgesia, i.e. hyperalgesia due to central sensitization, develops in this lesion.

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