

Selective Opiate Modulation of Nociceptive Processing in the Human Brain

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Casey, Kenneth L., Peter Svensson, Thomas J. Morrow, Jonathan Raz, Cyrenius Jone, and Satoshi Minoshima. Selective opiate modulation of nociceptive processing in the human brain. *J Neurophysiol* 84: 525–533, 2000. Fentanyl, a μ -opioid receptor agonist, produces analgesia while leaving vibrotactile sensation intact. We used positron emission tomography (PET) to study the mechanisms mediating this specific effect in healthy, right-handed human males (ages 18–28 yr). Subjects received either painful cold ($n = 11$) or painless vibratory ($n = 9$) stimulation before and after the intravenous injection of fentanyl (1.5 $\mu\text{g}/\text{kg}$) or placebo (saline). Compared with cool water (29°C), immersion of the hand in ice water (1°C) is painful and produces highly significant increases in regional cerebral blood flow (rCBF) within the contralateral second somatosensory (S2) and insular cortex, bilaterally in the thalamus and cerebellum, and medially in the cerebellar vermis. Responses just below the statistical threshold ($3.5 < Z < 4.0$) are seen in the contralateral anterior cingulate, ipsilateral insular cortex, and dorsal medial midbrain. The contralateral primary sensory cortex (S1) shows a trend of activation. Except for slight changes in intensity, this pattern is unchanged following a saline placebo injection. Fentanyl reduces the average visual analogue scale ratings of perceived pain intensity (47%) and unpleasantness (50%), reduces pain-related cardioacceleration, and has positive hedonic effects. After fentanyl, but not placebo, all cortical and subcortical responses to noxious cold are greatly reduced. Subtraction analysis [(innocuous water + fentanyl) – (innocuous water + no injection)] shows that fentanyl alone increases rCBF in the anterior cingulate cortex, particularly in the perigenual region. Vibration (compared with mock vibration) evokes highly significant rCBF responses in the contralateral S1 cortex in the baseline (no injection) and placebo conditions; borderline responses ($3.5 < Z < 4.0$) are detected also in the contralateral thalamus. Fentanyl has no effect on the perceived intensity or unpleasantness of vibratory stimulation, which continues to activate contralateral S1. Fentanyl alone [(mock vibration + fentanyl) – (mock vibration + no injection)] again produces highly significant activation of the perigenual and mid-anterior cingulate cortex. A specific comparison of volumes of interest, developed from activation peaks in the baseline condition (no injection), shows that fentanyl strongly attenuates both the contralateral thalamic and S1 cortical responses to noxious cold stimulation ($P < 0.048$ and 0.007 , respectively) but fails to affect significantly these responses to vibrotactile stimulation ($P > 0.26$ and 0.91 , respectively). In addition, fentanyl, compared with placebo, produces a unique activation of the mid-anterior cingulate cortex during fentanyl analgesia, suggesting that this region of the cingulate cortex participates actively in medi-

ating opioid analgesia. The results are consistent with a selective, fentanyl-mediated suppression of nociceptive spinothalamic transmission to the forebrain. This effect could be implemented directly at the spinal level, indirectly through cingulate corticofugal pathways, or by a combination of both mechanisms.

INTRODUCTION

Despite decades of research, we have a very limited understanding of the neural mechanisms mediating the analgesia produced by systemically administered opioids in humans. The pharmacology of the several opioid receptors has been elucidated (Fowler and Fraser 1994), and there is information about their relative distribution in the human nervous system (Pfeiffer et al. 1982). Positron emission tomography (PET) studies have revealed high levels of opioid receptor binding in the human anterior cingulate and prefrontal cortex (Jones et al. 1991b). Immunohistochemical studies have identified the μ -opioid receptor in the cerebral cortex, hippocampus, and striatum and on primary afferent fibers in the superficial dorsal horn of rat spinal cord (Arvidsson et al. 1995). Mechanistic studies using animal models have shown that systemic opioids can attenuate the responses of rostrally projecting spinal nociceptive neurons directly and, through the activation of descending supraspinal pathways, indirectly (Jensen 1997; Yaksh 1997).

The development of functional brain imaging now provides the opportunity to study the physiological action of opioids in the human CNS. Firestone et al. (1996) used PET imaging and Schlaepfer et al. (1998) used single photon emission computed tomography (SPECT) to demonstrate synaptically induced increases in regional cerebral blood flow (rCBF) in the human brain following the systemic administration of μ receptor agonist opioids. Both of these studies revealed increased activity in the anterior cingulate cortex, including the perigenual region. Adler et al. confirmed this result in an investigation of systemic analgesia induced by the μ receptor agonist fentanyl (Adler et al. 1997). However, none of these investigators demonstrated a specific analgesia-related reduction in rCBF responses to painful stimuli. Our PET study confirms the

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fentanyl-induced activation of anterior cingulate cortex but also shows that, in accord with clinical experience and psychophysical measurement, this analgesia is associated with a marked and selective reduction in all rCBF responses to noxious deep cold, but not vibrotactile, stimulation.

METHODS

Subjects

Twenty healthy right-handed males, ages 18–28 yr, gave informed consent to participate in this study. The consent form and the study protocol were approved by the Human Studies Committee of the Ann Arbor Veteran's Affairs Medical Center and by the Institutional Review Board for Human Studies at the University of Michigan Medical Center. Females were excluded because of evidence of gender differences in forebrain responses to noxious stimuli (Paulson et al. 1998).

Stimulation and psychophysical procedures

All participants received instruction in estimating the magnitude of perceived stimulus intensity and unpleasantness by using a visual analog scale (VAS) in which 0 equals no sensation (vibratory stimulation) or no pain (ice-water stimulation) and 10 is the most intense vibration (or pain) imaginable. An odor analogy was described to assist the subjects in differentiating ratings of stimulus unpleasantness from stimulus intensity.

Eleven subjects participated in the cold pain component of the study. Each immersed his left hand either in innocuous cool (29°C) or noxious cold (1°C) water 30 s before the onset of each scan and for the 60-s duration of the scan. Subjects were asked to remain silent and immobile, with eyes closed during each scan and, after each scan was completed, to describe the stimulus in their own words and to indicate their rating of stimulus intensity on the VAS. After each scan in the placebo and fentanyl conditions, subjects were asked to rate, on a 0–10 scale, their subjective feelings on each of 10 items derived from the study of Zacny et al. (1995).

Nine subjects participated in the vibratory part of the study. The hand-held vibrator (Model 91, Daito, Osaka, Japan) has a circular surface stimulation area of ~3 cm² and oscillates at a frequency of 130 Hz at an amplitude of 2 mm. Each subject received either vibration applied to the left volar forearm or mock vibration (vibrator held above the arm) during each scan. Otherwise instructions and conditions duplicated those applied to the group receiving the cold pain stimulus.

Positron emission tomography

We used a Siemens/CTI 931/08–12 scanner with 15 tomographic slices covering an axial field of view of 10 cm. A transmission scout view was used to position each subject in the scanner approximately parallel to the canthomeatal line. Head position was maintained by soft restraint and laser beam positioning on facial fiducial marks; head motion was corrected by a computer coregistration algorithm for each subject (Minoshima et al. 1993a,b). At least 15 min elapsed between each scan. For each scan, each subject received a 33-mCi intravenous bolus injection of H₂¹⁵O through an indwelling catheter in the right antecubital vein. Data acquisition began 5 s after the estimated arrival of radioactivity in the brain and continued for ~60 s. After normalizing each image set to whole brain counts, mean radioactivity concentration images estimating rCBF were created for each experimental condition by stereotactic anatomical standardization techniques (Minoshima et al. 1992, 1993a, 1994). Subtraction images were made for each subject by subtracting the images acquired during the lower intensity stimulation from those acquired during the highest intensity stimulation. A voxel-by-voxel statistical subtraction analysis (*Z* score)

with adjustment for multiple comparisons was performed by estimating the smoothness of subtraction images following three-dimensional Gaussian filtering (FWHM = 9 mm) to enhance signal-to-noise ratio and compensate for anatomical variance (Friston et al. 1991). Voxels showing a significantly increased CBF compared with the average noise variance computed across all voxels (pooled variance) were identified. The critical level of significance (*Z* = 4.0) was determined by adjusting *P* = 0.05 using this information (Adler and Hasofer 1976; Worsley et al. 1992). Only those voxels with normalized CBF values 60% of the global value were analyzed in this study.

To compare specifically the effect of fentanyl on the activation of the contralateral ventral posterior thalamus and the primary (S1) somatosensory cortex, an additional separate volume of interest (VOI) analysis was performed. We developed these VOIs from the coordinates of peak pain and vibratory activations obtained before either the placebo or fentanyl injections and applied them to the analysis of the placebo and fentanyl conditions of each experimental group (painful ice water or vibration). The size and shape of each VOI was determined separately for each of the above structures by employing a method similar to that described by Burton et al. (1993). The volume defined by these voxels was then progressively expanded in three dimensions to include only those contiguous voxels that showed rCBF increases that were significantly greater than the global mean change (*P* < 0.05, uncorrected for multiple comparisons). For purposes of comparison, the responses within each VOI are expressed as the average increase in rCBF within the volume of that VOI. To determine the statistical significance of rCBF increases, a paired *t* statistic was computed for each VOI from the average percentage increase in CBF across all subjects in each of the studies.

Procedure

Four scans, two during mock vibration or neutral water and two during vibration or ice water, were acquired during each of three conditions in the following order: baseline (stimulation only), placebo (intravenous saline injection), and fentanyl (1.5 µg/kg iv bolus). The order of stimulus presentation was randomized across subjects. Automated sphygmomanometric recordings of blood pressure were taken immediately after each of the placebo and fentanyl scans; heart rate, and percentage oxygen saturation were monitored continuously (Propac Encore Model 206EL, Protocol Systems, Beaverton, OR). Subjects were informed that two drugs were being tested. The injection of “drug 1” was announced before the set of placebo scans and “drug 2” before the set of fentanyl scans. Each injection was administered through an indwelling catheter in the left antecubital vein ~10 min before the first of the set of four scans (placebo or fentanyl). Venous blood samples (~7 ml) were taken from this catheter before the fentanyl injection and immediately after each fentanyl scan for radioimmuno assay analysis of fentanyl plasma levels (Research Diagnostics, Flanders, NJ). (Chapman et al. 1990). To assure accuracy of the blood sample, 5 ml of blood was drawn and discarded before each sample. Blood sampling was simulated during the placebo scans by manipulating syringes attached to the sampling line outside the subject's direct line of vision.

Statistical analysis

A repeated-measures (mixed model) ANOVA was used to determine the effect of each stimulus condition (baseline, placebo, fentanyl) on each of the autonomic variables (blood pressure, heart rate, percentage oxygen saturation) and on the VAS ratings of stimulus intensity and unpleasantness. A similar separate analysis was performed to examine the effect of fentanyl plasma level on each of the above variables. Parametric and nonparametric (Kruskal-Wallis) ANOVAs were used to determine specifically the effect of scan sequence on plasma fentanyl levels and on VAS ratings of pain intensity during the fentanyl condition. Paired *t*-tests were used to

determine the effects of fentanyl and placebo on the S1 cortical and contralateral thalamic rCBF responses to painful ice water and vibration. The Wilcoxon signed-rank test was used to determine the significance of changes in subjective feelings. Linear correlation analysis and one-way ANOVA was used to test the relationship, across all subjects, between rCBF in each VOI and the degree of fentanyl-induced analgesia or positive hedonic effect (see following text). In this study, we could not perform enough repeated scans during identical stimulus-drug conditions to permit a within-subject correlative analysis of rCBF with other parameters.

RESULTS

Effect of fentanyl alone and on pain-evoked supraspinal activity (voxel-by-voxel analysis)

Subtracting the effect of cool water from ice-water stimulation in the baseline (no injection) condition reveals highly significant rCBF increases contralaterally within the S2 and insular cortex, bilaterally in the thalamus and cerebellum, and medially in the cerebellar vermis. Responses just below the statistical threshold ($3.5 < Z < 4.0$) are detected in the contralateral anterior cingulate and ipsilateral insular cortex and in the dorsal medial midbrain (Table 1). A trend of activation is seen in the contralateral S1. Except for slight regional changes in the intensity of response, this overall pattern is unchanged following a saline placebo injection (Table 2 and Fig. 1). Following the fentanyl injection, however, subtraction analysis

TABLE 1. *rCBF responses to ice water immersion during the baseline condition*

Structure	Coordinates (x, y, z)	Percent Change in rCBF	Z Score
Contralateral			
Insula/lenticular nucleus	-26, 3, 11	5.8	5.0
VL thalamus	-12, -6, 9	5.6	4.8
S2 cortex	-35, -17, 20	5.3	4.5
Cerebellum (hemisphere)	-26, -64, -22	5.2	4.5
Medial thalamus	-6, -22, 14	4.9	4.2
(anterior cingulate cortex; B24)	-8, 12, 29	4.0	3.5
S1 cortex	-35, -26, 56	3.6	2.8
Ipsilateral			
Cerebellum			
Paravermis	17, -58, -16	8.0	6.9
Hemisphere	35, -55, -32	7.1	5.2
VPL thalamus (mid-insula)	10, -15, 9	4.8	4.1
	26, 5, 11	4.1	3.6
Midline			
Cerebellum			
Vermis	-3, -58, -20	6.2	5.4
Dorsal midbrain	-3, -26, -4	4.1	3.6

Structures showing voxels with statistically significant ($Z > 4.0$) or borderline ($3.5 < Z < 4.0$) peak rCBF increases (% Δ rCBF), compared to normalized global cerebral blood flow, during 90 s of tonic cold pain (immersion of the left hand in 1°C water) compared to innocuous cool stimulation (immersion of the left hand in 29°C water) during the baseline (no placebo or fentanyl) condition. Structures showing borderline responses are listed in parentheses; the response in the primary (S1) somatosensory cortex, a structure of interest, is also listed parenthetically. Stereotactic coordinates (mm) of peak voxels are listed in reference to the midline (x, right negative), anterior commissure (y, anterior positive), and the horizontal plane connecting the anterior and posterior commissures (z, superior positive) according to the atlas of Talairach and Tournoux (1988). VL, ventrolateral thalamus; S2, secondary somatosensory; VPL, ventral posterolateral thalamus.

TABLE 2. *rCBF responses to ice water immersion stimulation during the placebo condition*

Structure	Coordinates (x, y, z)	Percent Change in rCBF	Z Score
Contralateral			
VPL thalamus	-17, -17, 9	6.0	5.5
Cerebellum (hemisphere)	-37, -62, -25	5.2	4.8
Ant. cingulate ctx; B24	-17, 44, 4	4.6	4.3
Anterior insula	-26, 17, 14	3.9	3.6
S2 cortex	-33, -31, 20	3.5	3.2
S1 cortex	-28, -22, 56	3.5	3.2
Ipsilateral			
Cerebellum (paravermis)	10, -55, -16	8.5	7.9
VPL thalamus	10, -10, 7	6.6	6.1
Cerebellum	12, -78, -34	7.5	5.9
Hemisphere			
Anterior insula	28, -4, 18	4.1	3.7
Midline			
Cerebellum (vermis)/dorsal midbrain			
	1, -44, -18	6.8	6.3
Ant. cingulate ctx; B24	-3, -1, 36	4.6	4.2

Same as Table 1 except that the water-immersion stimulation is applied during the placebo condition.

shows that, except for borderline responses in the contralateral thalamus, the ipsilateral cerebellum, and the cerebellar vermis, all cortical and subcortical pain-related activations are reduced well below statistical significance (Table 3).

To determine if any forebrain structures are activated by fentanyl alone in this group of subjects, we subtracted the effect of the baseline condition (no injection) from the effect of fentanyl during innocuous water stimulation [(innocuous water + fentanyl) - (innocuous water + no injection)]. This analysis, which eliminates interactions between fentanyl and pain or placebo effect, shows that fentanyl alone produces highly significant responses ($Z = 5.8-4.1$; average 6.4% increase in rCBF) bilaterally in the perigenual ($x = \pm 3$; $y = 30$; $z = 7$) and mid-anterior ($x = 8, -6$; $y = 1, 8$; $z = 43, 27$) cingulate cortex during the control innocuous stimulation (Fig. 1). Activation of the ipsilateral S2 cortex ($Z = 4.7$), superior temporal gyrus ($Z = 4.0$), and occipital gyrus ($Z = 4.8-4.5$) is also observed. Similar results are obtained when the fentanyl and baseline or placebo effects are compared during ice-water stimulation except that the S2 cortex is not activated.

Effect of fentanyl alone and on vibration-evoked supraspinal activity (voxel-by-voxel analysis)

Vibration (minus the effect of mock vibration) evokes highly significant rCBF responses in the contralateral S1 cortex in the baseline (no injection) condition. Borderline responses ($3.5 < Z < 4.0$) are detected also in the medial contralateral thalamus, S2 cortex, and cerebellum (Fig. 2, Table 4). Strong contralateral S1 and S2 cortical responses ($Z = 4.7$ and 4.5, respectively) and sub-significant ($Z = 3.12$) contralateral thalamic responses are present following the placebo injection. After the injection of fentanyl, contralateral S1 responses to vibratory stimulation persist ($Z = 4.4$, average 5.0% increase in rCBF); activation is also present in the contralateral lenticular nucleus.

Fentanyl alone [(mock vibration + fentanyl) - (mock vibration + no injection)] can again be shown to produce highly significant and bilateral activation of the perigenual ($x = 12$,

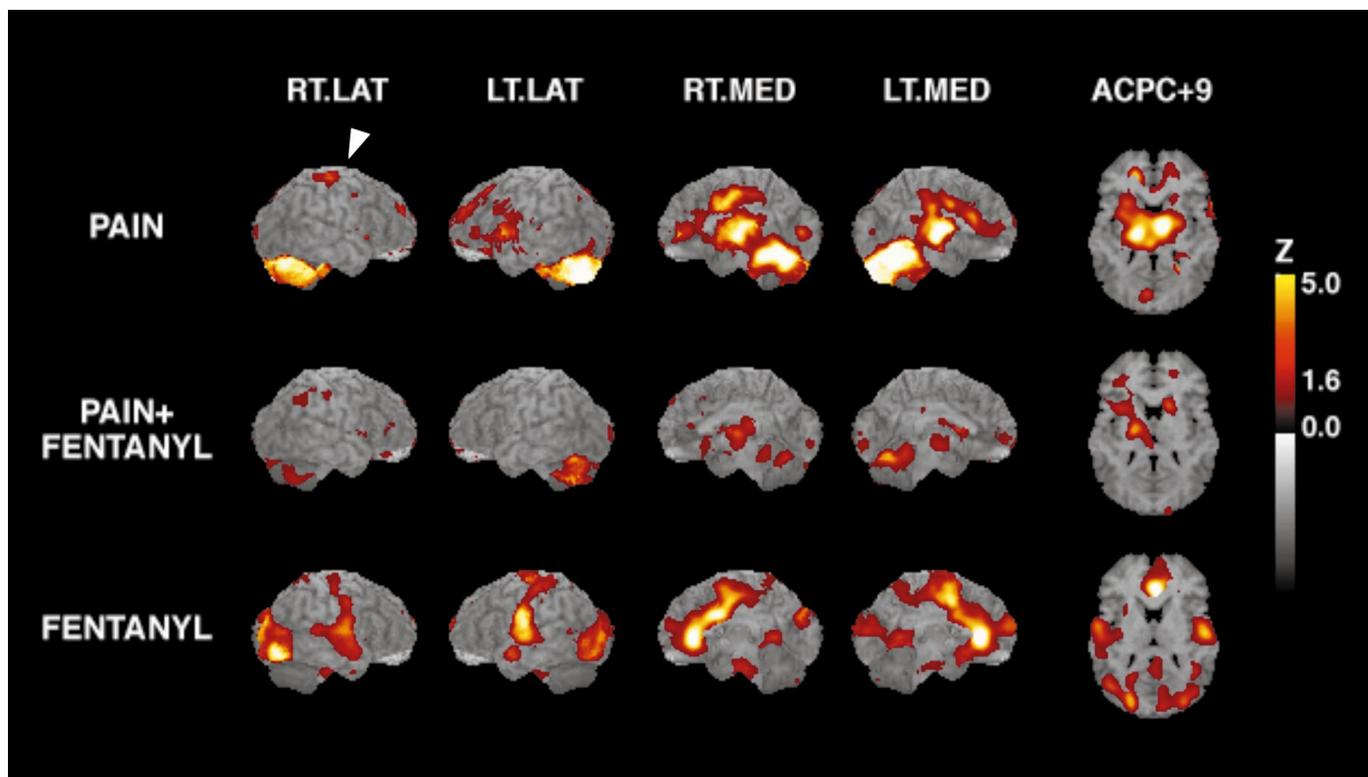


FIG. 1. Regional cerebral blood flow (rCBF) increases (11 subjects) while the left hand is immersed in painfully cold (1°C) water compared with painless cool (29°C) water (*top 2 rows*). Averaged subtraction responses are superimposed on surface-rendered and transverse images of a magnetic resonance imaging (MRI) brain scan anatomically transformed to conform to stereotactic atlas coordinates³⁴. Right (R) and left (L) medial (MED) and lateral (LAT) views of the hemispheres are shown. Transverse images, with right hemisphere to the left, are shown 9 mm superior to a plane joining the anterior (AC) and posterior (PC) commissures. *Top*: placebo condition [(placebo + ice water) [minus] (placebo + innocuous water)] (see Table 2). Note activation of primary sensory cortex (S1) in right lateral hemisphere (▲). *Middle*: same as above except during fentanyl analgesia [(fentanyl + ice water) – (fentanyl + innocuous water)]. There is no significant cortical activation (see Table 3). *Bottom*: effect of fentanyl alone during innocuous water stimulation [(fentanyl + innocuous water) – (no injection + innocuous water)]. Note highly significant bilateral activation of the perigenual and mid-cingulate cortex

–6; $y = 35$; $z = 14, 9$) and mid-anterior ($x = 10, -10$; $y = 8, 10$; $z = 34, 27$) cingulate cortex during, and in the absence of, vibratory stimulation ($4.1 < Z < 6.2$; Fig. 2).

Plasma levels of fentanyl

Plasma fentanyl levels average 0.437 ± 0.086 (SD) ng/ml throughout the study and range from 1.10 to 0.11 ng/ml for the 9th–12th scan across all subjects. Neither intra- nor intersubject variations in plasma fentanyl levels can be shown to affect any outcome variable. The average plasma fentanyl level de-

clines during the four scans obtained during hypoalgesia, but the VAS pain ratings of the subjects in the ice water part of this study are unaffected (Fig. 3).

Differential effect of fentanyl on forebrain mechanisms mediating noxious and innocuous sensations (VOI analysis)

To compare directly the effect of fentanyl on the forebrain processing of painful cold and vibrotactile stimuli, it is necessary to examine the rCBF responses within identical cerebral locations during each experimental condition in each group of subjects. Accordingly, we developed specific VOI (Burton et al. 1993) from activation peaks in the contralateral S1 cortex and thalamus of each group of subjects during the baseline condition (cold pain or vibration but no injection). These VOIs were then applied to the subtraction images of individuals in each group during the placebo and fentanyl conditions. Paired *t*-tests (2-tailed) were used to detect significant differences in the rCBF responses within these VOIs. As shown in Fig. 4, painful ice water and vibration each produces rCBF responses within these VOIs. However, fentanyl strongly attenuates both the contralateral thalamic and S1 cortical responses to noxious cold stimulation ($P < 0.048$ and 0.007 , respectively) but fails to affect significantly these responses during vibrotactile stimulation ($P > 0.26$ and 0.91 , respectively).

TABLE 3. rCBF responses to ice water immersion stimulation during the fentanyl condition

Structure	Coordinates (x, y, z)	Percent Change in rCBF	Z Score
Contralateral (VPL thalamus)	–19, –13, 9	4.0	3.6
Ipsilateral Cerebellum; hemisphere	33, –58, –34	5.5	3.6
Midline Cerebellum; vermis	6, –62, –20	4.2	3.8

Same as Table 1 except that the ice water immersion stimulation is applied during the fentanyl condition.

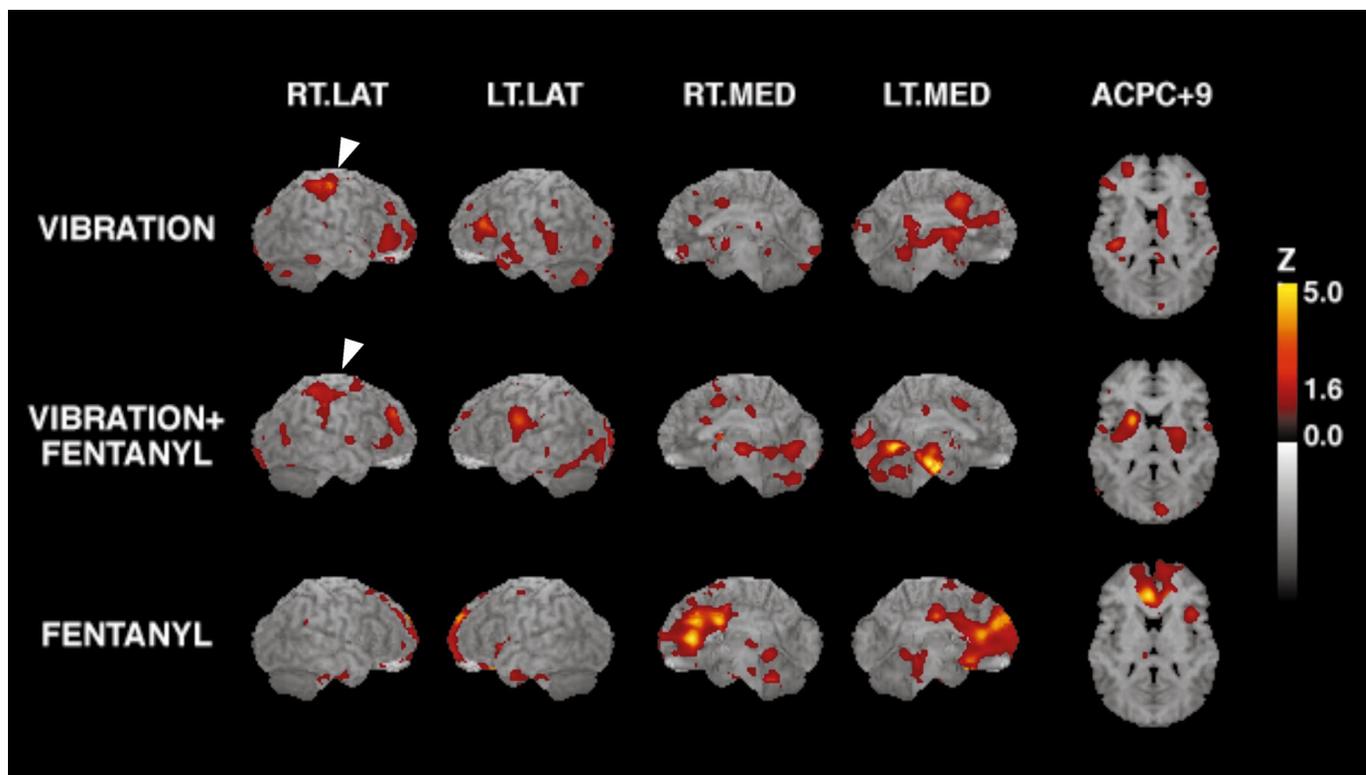


FIG. 2. Same as Fig. 1 except showing rCBF increases (9 subjects) during vibratory, compared with mock, stimulation of left forearm. *Top*: vibratory stimulation during the placebo condition [(placebo + vibration) – (placebo + mock vibration)] (see Table 4). Note activation of S1 cortex in right lateral hemisphere (\blacktriangle). *Middle*: same as preceding row except during fentanyl analgesia [(fentanyl + vibration) – (fentanyl + mock vibration)]. Note persistence of S1 cortical activation on the right (\blacktriangle). *Bottom*: effect of fentanyl alone during mock vibratory stimulation [(fentanyl + mock vibration) – (no injection + mock vibration)]. Note highly significant bilateral activation of the perigenual and midcingulate cortex.

To determine which supraspinal structures might participate actively in mediating the analgesic effect of fentanyl, we developed specific VOIs (Burton et al. 1993) based on the results of our previous pain activation studies (Casey et al. 1994, 1996) and identified from peaks of activation ($Z > 3.4$) produced by painful, as compared with painless, cold water during the baseline condition (no placebo or fentanyl; see coordinates, Table 1). We applied these pain-activated VOIs to the placebo and fentanyl conditions during painful ice-water immersion to reveal those structures activated specifically by fentanyl during fentanyl analgesia. Only 1 of 11 VOIs, located in the mid-anterior cingulate cortex, showed an increased rCBF

during fentanyl analgesia (Fig. 5). All other structures analyzed had reduced rCBF, compared with placebo in this condition. No VOI response (% change from placebo) correlated with the analgesic effect of fentanyl (measured as % change in VAS score; linear correlation analysis across subjects). However, a one-way ANOVA revealed a significant difference among these responses ($P < 0.001$). Post hoc multiple pair-wise comparisons revealed that this anterior cingulate response was different from all others ($P: 0.01$ – 0.001) except for the bilateral insula, contralateral S2 cortex, and the medial dorsal midbrain.

TABLE 4. rCBF responses to vibratory stimulation during the baseline condition

Structure	Coordinates (x, y, z)	Percent Change in rCBF	Z Score
Contralateral			
S1 cortex	-37, -26, 45	5.7	4.5
Medial thalamus	-3, -17, 4	4.7	3.7
Posterior insula	-37, -13, 16	4.7	3.8
Cerebellum, paravermis	-10, -51, -32	5.7	3.7
Ipsilateral			
Mesial premotor cortex (SMA, B6)	8, 21, 52	4.5	3.6

Same as Table 1 except that the responses to vibratory, as compared to mock, stimulation during the baseline (no injection) condition are listed.

Subjective effects of fentanyl

Fentanyl reduces the average VAS ratings of perceived pain intensity (mean \pm SD) from 6.26 ± 1.41 (baseline) and 6.40 ± 1.31 (placebo) to 3.36 ± 1.15 . Perceived unpleasantness is similarly affected (baseline: 6.63 ± 1.33 ; placebo: 6.54 ± 1.31 ; fentanyl: 3.30 ± 1.14). A repeated-measures ANOVA (mixed model) of the intensity and unpleasantness rating differences between ice and neutral water reveals highly significant effects of the fentanyl, but not of the placebo, on both measures ($P = 0.0001$). Fentanyl has no effect on the ratings of perceived vibratory intensity (baseline: 3.42 ± 2.22 ; placebo: 3.30 ± 2.05 ; fentanyl: 3.07 ± 2.14) or unpleasantness (baseline: 1.35 ± 1.30 ; placebo: 1.51 ± 1.49 ; fentanyl: 1.23 ± 1.68).

Compared with the placebo condition, fentanyl produces

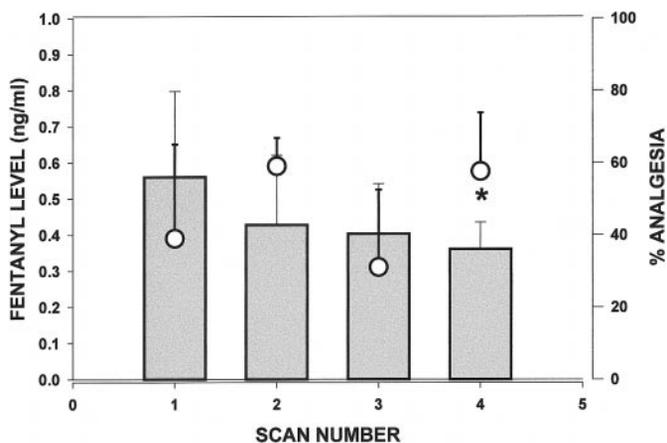


FIG. 3. Bars show the mean (\pm SD) plasma levels of fentanyl (ng/ml) of all subjects ($n = 20$) immediately following each of the 4 PET scans obtained after the intravenous injection of fentanyl ($1.5 \mu\text{g}/\text{kg}$) ~ 10 min before scan 1 of this set. Both parametric and nonparametric (Kruskal-Wallis) ANOVAs with posthoc comparisons (Tukey, Dunn) show that the plasma fentanyl level during scan 4 is significantly lower than that during scan 1 ($P = 0.012$ and 0.022 , respectively). However, the mean (\pm SD) percentage analgesia [(VAS during placebo - VAS during fentanyl)/VAS during placebo $\times 100$] experienced by the subjects participating in the ice-water stimulation study (\circ) was not significantly reduced during this set of scans ($P > 0.05$; parametric and nonparametric ANOVAs).

increases in pleasant body sensations and thoughts; feelings of being carefree, sedated, and a loss of body control during all stimulation conditions ($0.002 < P < 0.03$) (Fig. 6). We again performed a comparison of responses within the 11 previously identified VOIs to determine if any structures were activated by fentanyl, compared with placebo, during innocuous stimulation and thus possibly related to the positive hedonic effects of fentanyl rather than the analgesia. No VOI response (% change from placebo) correlated with the positive hedonic effect of fentanyl (total fentanyl-placebo feeling state score increase across the above 4 categories; linear correlation analysis across subjects). Moreover, a one-way ANOVA failed to reveal any

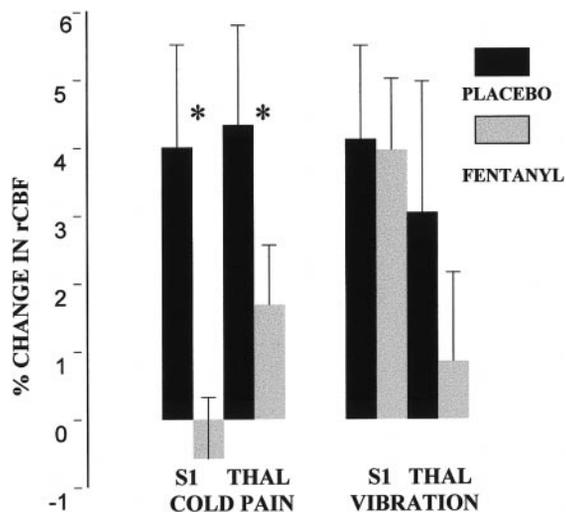
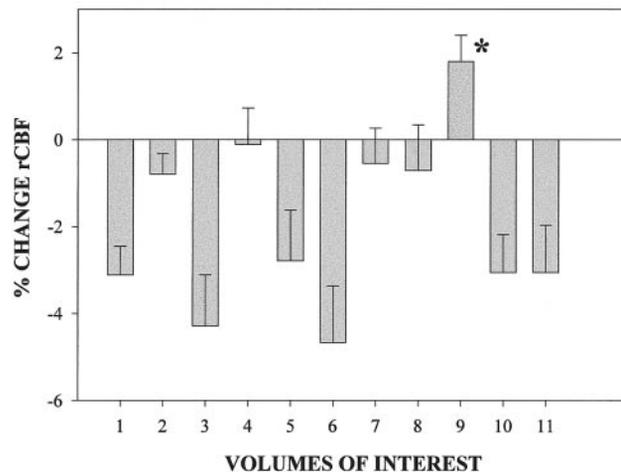


FIG. 4. Volume of interest (VOI) analysis (see METHODS). During fentanyl analgesia (\square) but not during the placebo condition (\blacksquare), the mean (\pm SE) percentage increase in rCBF during cold pain is significantly suppressed (*) in the S1 cortex ($P < 0.048$) and thalamus ($P < 0.007$). The responses of these same VOI to vibratory stimulation, however, are not significantly affected ($P > 0.26$ and 0.91 , respectively). The thalamic response during vibration is highly variable.

FENTANYL EFFECT DURING PAIN



1=cerebellum (paravermal) 2=contra. insula 3=contra. VP thalamus
4=contra. S2 cortex 5=contra. medial thalamus 6=ipsi. VP thalamus
7=ipsi. mid-insula 8=dorsal midbrain 9=contra. ant. cingulate
10=contra. S1 cortex 11=contra. M1/S1 cortex

FIG. 5. Responses of VOI to fentanyl, compared with placebo, during ice-water immersion stimulation, when subjects were analgesic. Only the contralateral anterior cingulate cortex had a fentanyl-associated rCBF increase (*, $P < 0.01$). The VOIs were chosen based on previous pain activation studies and developed (see text) from peaks of activation ($Z > 3.4$) during ice-water immersion, compared with innocuous water, in the baseline condition (no placebo or fentanyl). These baseline, pain-activated VOIs were then applied to the placebo and fentanyl conditions, and the rCBF responses to fentanyl calculated as % rCBF change were compared with placebo [(fentanyl-placebo)/placebo $\times 100$].

significant differences among the responses within VOI in this condition.

Autonomic effects of fentanyl

Immersion of the hand in the ice water increases the average (\pm SD) heart rate from 59.1 ± 2.9 to 71.2 ± 2.6 beats/min. Following the fentanyl injection, ice-water stimulation causes the average heart rate to increase from 59.1 ± 3.2 to 63.1 ± 3.3

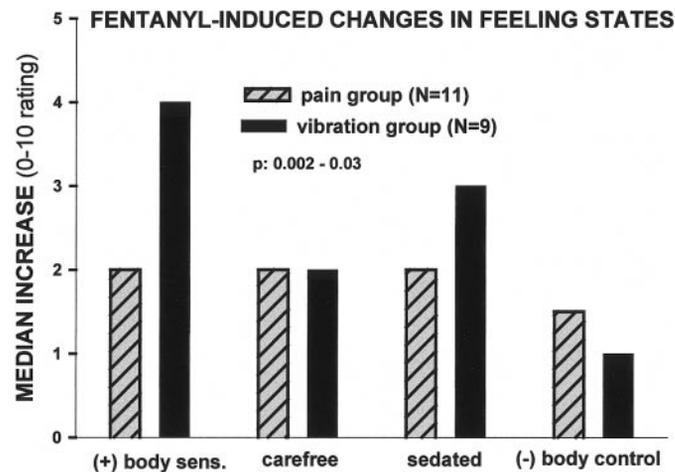


FIG. 6. There was a significant increase in the median verbal numerical ratings of 4 of 10 feeling states that were sampled (pleasant body sensations, feelings of being carefree, feeling sedated, and loss of body control) following fentanyl, compared with the placebo, injection (Wilcoxon signed rank test).

beats/min. A repeated-measures ANOVA (mixed model) of the individual responses reveals a significant heart rate response to ice-water stimulation over all conditions ($P = 0.014$) and a highly significant effect of fentanyl in reducing this response compared with the baseline or placebo conditions ($P = 0.001$).

Blood pressure readings were obtained from the arm used for stimulation and therefore could be obtained only before and after stimulation periods. Nonetheless, we detected significant increases in both systolic and diastolic average blood pressures (mmHg) across all conditions following ice water, but not neutral water, stimulation (baseline: 132/57 increasing to 143/63; placebo: 135/58 increasing to 139/65; fentanyl: 134/56 increasing to 138/61). A repeated-measures ANOVA (mixed model) of the individual responses does not reveal an effect of fentanyl on these blood pressure increases. Vibratory stimulation has no effect on blood pressure.

A comparison of optical transcutaneous measurements of the percentage of blood oxygen saturation taken before and after the administration of fentanyl reveals a slight but statistically significant decrease across all subjects (average \pm SD) $99.32 \pm 0.34\%$ to $97.27 \pm 0.66\%$. ($P = 0.0039$) immediately following the administration of fentanyl.

DISCUSSION

The cortical and thalamic responses to noxious cold stimulation are similar to those observed in our previous studies (Casey et al. 1994, 1996) except that premotor and prefrontal activation is below the statistical significance level in this study. Fentanyl, a μ -opioid receptor agonist, suppresses these pain-evoked responses during hypoalgesia. Presumably, the mild pain sensation that remains following fentanyl is mediated by the activity of cortical and thalamic neurons that falls below the level of significance established for this study (Fig. 1; Table 3).

Our experiment demonstrates a neural basis for the selective hypoalgesic effect of fentanyl because the cortical and thalamic responses to painless vibratory stimulation are spared following hypoalgesic doses of this drug (Fig. 4). This finding is in accord with the observation that opioid analgesia spares vibrotactile perception (Wikler et al. 1945). The mechanism for achieving this selective analgesic effect is unknown. Fentanyl may have a relatively selective effect on cortical, as compared with thalamic, nociceptive responses. Although the S1 responses were nearly equal, fentanyl eliminated the S1 response to painful cold while sparing completely the S1 response to vibration. Compared with this highly selective cortical action, the effect of fentanyl on thalamic responses was similar during both stimulus conditions. The thalamic responses were slightly smaller and more variable, so the effect of fentanyl was statistically different but of comparable magnitude. However, it is likely that given the density of modality representations in the thalamus and the spatial resolution of functional brain imaging we are better able to observe modality-specific effects at the cortical level.

Given the background of available evidence, our results suggest at least three major possibilities for the hypoalgesic effect of fentanyl: a direct and selective attenuation of the nociceptive responses of spinothalamic tract neurons in the dorsal horn of the spinal cord, selective suppression of spinothalamic neuronal responses by the excitation of corticofugal

neurons in the anterior cingulate cortex, and a combination of the first and second mechanisms. Yaksh (1997) has recently reviewed evidence that the systemic administration of opiates directly and selectively suppresses the nociceptive excitation of spinal cord dorsal horn neurons in experimental animals and humans. Overall, the observations leave little doubt that at least some of the analgesia produced by systemically administered opioids is due to a direct and selective suppression of nociceptive excitation at the spinal cord level. Our results are consistent with this mechanism because fentanyl selectively suppresses the nociceptive activation of those brain stem, thalamic, and cortical regions that have been shown to respond differentially to noxious stimuli (Casey et al. 1994, 1996; Coghill et al. 1994; Craig et al. 1996; Jones et al. 1991a; Talbot et al. 1991) while sparing the physiologically and anatomically distinct vibrotactile pathways (Coghill et al. 1994).

There is also compelling evidence that supraspinal mechanisms could mediate the analgesia produced by systemically administered opioids (Jensen 1997; Yaksh 1997). In rodents, morphine-induced antinociception is attenuated by lesions or local anesthetic injections within the medial medulla (Proudfit 1980; Proudfit and Anderson 1975) or lesions in the central nucleus of the amygdala (Manning and Mayer 1995a,b). In addition, microinjection of the opioid receptor antagonist naloxone into the midbrain periaqueductal gray (PAG) or posterior hypothalamus reverses the analgesia of systemic morphine as measured in the rat formalin test (Manning and Franklin 1998). Our results also support the possibility that supraspinal structures participate in mediating opioid analgesia because we found that, among the VOIs we examined, the mid-anterior cingulate was unique in showing an increased rCBF during fentanyl analgesia (Fig. 5).

There is anatomical (Mantyh 1982; Room et al. 1985) and neuropharmacological (Jones et al. 1991b; Lewis et al. 1983) evidence that the PAG, which has long been considered an important mediator of analgesia (Basbaum and Fields 1984), could be excited by corticobulbar neurons in the anterior cingulate cortex. The evidence suggests that the fentanyl-induced activation of the cingulate cortex could excite, by disinhibition or direct excitation, a descending cascade of analgesic mechanisms mediated through the PAG. However, although we detect activation of the dorsomedial midbrain (in the region of the PAG) during ice-water stimulation, we detect only very weak dorsomedial midbrain activity ($Z = 1.5$; rCBF increase of 1.9%) at nearly the same stereotactic coordinates ($x, -3$; $y, -28$; $z, -7$) when the effect of fentanyl alone is assessed during innocuous stimulation. No response is detected in this region during nociceptive stimulation in the fentanyl condition or when the effect of fentanyl alone is assessed during noxious stimulation. Even when the combined effect of fentanyl and ice-water stimulation is assessed by subtraction analysis [(ice water + fentanyl) - (innocuous water + no injection)], we cannot detect dorsomedial midbrain activity. Overall, the results suggest that the activation of descending brain stem mechanisms may not be as important a component of opioid analgesia in humans as it is in the rodent. Perhaps the activation of corticospinal projections from the anterior cingulate gyrus could attenuate spinothalamic responsiveness directly without involving brainstem structures (Hutchins et al. 1988; Luppino et al. 1994; Ralston and Ralston 1985). However, it is possible that the PET methods we used cannot detect a synap-

tically induced rCBF response to fentanyl in the dorsomedial midbrain. An additional possibility is that the dorsal midbrain activation we have seen in these and in other PET studies reflects the activity of nociceptive neurons in the superior colliculus (Redgrave et al. 1996a,b).

Comparing the cingulate cortical activity during pain with that during fentanyl alone may provide some insight into the physiological significance of the cingulate response to fentanyl. The mid-cingulate region is activated during both fentanyl alone and during pain (Figs. 1 and 2). However, one obvious difference is the intense activation of the most rostroventral perigenual cingulate during innocuous water stimulation (Fig. 1) or mock vibratory stimulation (Fig. 2) in the fentanyl condition. Anatomical and physiological studies suggest that mid-cingulate cortical activity is associated with higher order motor functions, such as response selection, while the rostroventral perigenual cortex may mediate autonomic responses and their associated affective experiences (Derbyshire et al. 1998; Ketter et al. 1996; Vogt et al. 1992, 1993). Our investigation now suggests further that the mid-anterior region of the cingulate cortex participates actively in mediating opioid analgesia.

The fentanyl-activated rostroventral perigenual region and the pain-activated mid-cingulate cortex are each adjacent to the rostral anterior cingulate region recently identified as encoding degrees of pain unpleasantness (Rainville et al. 1997). Taken together, these observations suggest that the reduced cardioacceleratory responses and pleasurable feelings experienced by all our subjects during the fentanyl condition are mediated through the activation of opiate-responsive mechanisms in the perigenual cingulate cortex. Whether these effects are independent accompaniments or contributing components of systemic opioid analgesia in humans remains to be determined.

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