Universiteit
Leiden
The Netherlands

Opioid therapy : a trade-off between opioid-analgesia and opioid-induced respiratory depression

Boom, M.C.A.

Citation

Boom, M. C. A. (2013, December 3). *Opioid therapy : a trade-off between opioid-analgesia and opioid-induced respiratory depression*. Department of Anesthesiology, Faculty of Medicine / Leiden University medical Center (LUMC), Leiden University. Retrieved from https://hdl.handle.net/1887/22623

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/22623

Note: To cite this publication please use the final published version (if applicable).

Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/22623 holds various files of this Leiden University dissertation

Author: Boom, Maria Catharina Anna
Title: Opioid therapy : a trade-off between opioid-analgesia and opioid-induced respiratory depression
Issue Date: 2013-12-03

CHAPTER 6

OPIOID CHRONOPHARMACOLOGY: INFLUENCE OF TIMING OF INFUSION ON FENTANYL'S ANALGESIC EFFICACY

M.C.A. Boom MD, Joost Grefkens MD, Eveline van Dorp MD PhD,
Erik Olofsen MSc. Gertjan Lourenssen BSc, Leon Aarts MD PhD,
Albert Dahan MD PhD, Elise Sarton MD PhD

Journal of Pain Research 2010; Sep 21;3:183-90

6.1 INTRODUCTION

Chronopharmacology studies the effect of the timing of drug administration (in terms of the hour in a 24 h period, the day in a 1 month or 1 year period, or the year in a life-time) on the drug's pharmacokinetics and/or pharmacodynamics.[1,2] When applied to the 24 h circadian rhythm, it is now known that numerous drugs exhibit a differential response depending on the time of administration. This also applies to drugs used in anesthesia such as local anesthetics, barbiturates, muscle relaxants and opioids.[1-2] For opioids, circadian effects have been observed for drug disposition (*e.g.*, meperidine and morphine) and therapeutic sensitivity (*e.g.*, tramadol and codeine).[2,4] However, the number of studies on opioid pharmacology is restricted and hence knowledge on the influence of the circadian rhythm on opioid analgesic efficacy remains poor.[2] Evidently further understanding and application of a chronotherapeutic approach to opioid treatment of acute and chronic pain would increase opioid efficacy and possibly improve the efficacy-safety balance.

To scrutinize the hypothesis that opioids display a diurnal antinociceptive effect, we performed a study on the influence of four distinct timing moments on fentanyl-induced analgesia in healthy volunteers. The analgesic effect of intravenous fentanyl, administered at 8 AM, 2 PM, 8 PM or 2 AM, was examined using an experimental heat pain model.

6.2 METHODS

6.2.1 SUBJECTS

Following approval of the protocol by the Leiden University Human Ethics Committee sixteen healthy volunteers (age 18-30, BMI < 28 $kg.m^{-2}$, 12 women, 4 men) were enrolled in the study. The study was registered at trialregister.nl (#NTR1254). Written and oral informed consent were obtained prior to the inclusion in the study. Exclusion criteria were: age < 18 years, body mass index > 30 $kg.m^{-2}$, presence of underlying disease, history of drug allergy, history of psychiatric disease, history of illicit substance abuse. All female subjects were taking oral contraceptives. The subjects were instructed not to eat or drink for at least 6 h before the study.

6.2.2 DESIGN

The subjects were randomly divided into two experimental groups. The first group received fentanyl at 2 PM and 2 AM; the second group at 8 AM and 8 PM. The experimental days were separated by a two week washout period. We studied two distinct groups in order to reduce the number of occasions at which the healthy volunteers received a potent opioid. At the appropriate time 2.1 $\mu g.kg^{-1}$ intravenous fentanyl was administrated intravenously over 90 s. Subsequently, heat pain measurements were taken every 10 minutes for 3 hours (first pain test at10 min after the start of the fentanyl infusion). Additionally, at each testing interval a Verbal Rating Score of sedation using a scale ranging from 0 to10 (0 = fully alert – 10 = severely sedated and sleepy) and end-tidal carbon dioxide measurements were

obtained via a face mask connected to a gas monitor (Multicap, Datex, Helsinki). Arterial hemoglobin oxygen saturation was measured via a finger probe (SpO_2) with a Masimo pulse oximeter (Irvine, CA). The study was powered to observe a 1 cm difference in visual analogue score (VAS) of a 10 cm scale ranging from 0 (= no pain) to 10 (= most intense pain imaginable) between two study groups (power = 90%, alpha = 0.05).

6.2.3 MESUREMENTS

Heat pain was induced using the TSA-II Neurosensory Analyzer (Medoc Ltd., Ramat Yishai, Israel). Using a 3 cm^2 probe, the skin on the volar side of the left or right forearm was stimulated with a gradually increasing stimulus (0.5 $^{o}C.s^{-1}$; baseline temperature 32 ^{o}C). Following heat stimulation, the subjects scored their VAS pain intensity on a 10 cm long scorecard. The thermode peak temperature depended on an initial trial phase in which the subject rated the pain to three peak temperatures: 46, 48 and 49 ^{o}C. The lowest stimulus causing a VAS > 5 cm was used in the remainder of the study. The test data were discarded. Next, baseline values (i.e., pre drug VAS) were obtained. The volar side of the arm was divided into six zones and marked as previously described.[5] The thermode was moved from zone to zone between stimuli to avoid sensitization to heat stimulus.

6.2.4 PHARMACOKINETIC-PHARMACODYNAMIC ANALYSIS

A linear mixed model was used to compare the baseline parameter values (thermode temperature to reach a VAS > 5, sedation score and end-tidal CO_2) using SPSS 16.0. (Chicago, IL). *P*-values < 0.05 were considered significant. In order to quantify the effect of fentanyl on pain relief we initially assessed the effect relative to baseline (*i.e.* ΔVAS, by subtraction of baseline VAS at each time point), and subsequently we calculated the area between the VAS data points and the zero-line (area between the effect-time curves, AEC). Consequently, a more negative the AEC means a higher analgesic the response. We present the AEC data as mean change in VAS over time (*i.e.*, AEC/180 min, unit = cm). Next, to get an indication of the presence of a circadian effect on fentanyl analgesia, the data were modeled using a sinusoid function:

$$AEC(t) = offset + A.\sin(2\pi ft + \varphi) \qquad \text{(eqn. 1)}$$

where the A = amplitude, f = frequency (occurrence of the sinus per 24 h) and φ = a phase shift. To obtain the 95% confidence interval of the sinusoid a bootstrap analysis was performed using 1000 reiterations with replacement. Data analysis was performed using the statistical package NONMEM version VI (ICON Development Solutions, Ellicott City, MD).[6] Similar procedures were performed for sedation and end-tidal CO_2.

6.3 RESULTS

No differences in baseline parameters were observed with-in group or between groups (see table 1). In each group there were 6 women and 2 men. All subjects completed the study without major side effects. Incidental occurrences of low SpO_2 (< 95%) were treated by prompting the subject to take a deep breath.

All injections were performed at the planned time of day ± 4.3 min (maximal range; not significant different between groups). After injection, all volunteers reached maximal analgesia within 20 min and returned to within 10% of their baseline pain sensitivity levels by the end of the experiment. The influence of the time of infusion on ΔVAS is shown in Fig. 1 and 2. Time-related variations are observed for peak analgesic effect (with the least effect at 2 AM), duration of effect (with the shortest duration at 8 AM) and the occurrence of a small hyperalgesic response (most pronounced at 2 AM). Individual AEC values (all divided over 180 min giving the mean change in VAS over 180 min) versus study time are given in Fig. 3 together with the data fit (± 95% confidence interval). A significant sinus wave was present in the data (the wave was significantly different from a linear response line, $P < 0.01$). Parameter values are: offset = –0.63 ± 0.25 cm, A = 0.65 ± 0.20 cm, φ = 27 ± 21 degrees (all parameters $P < 0.01$, values are typical value ± SE).

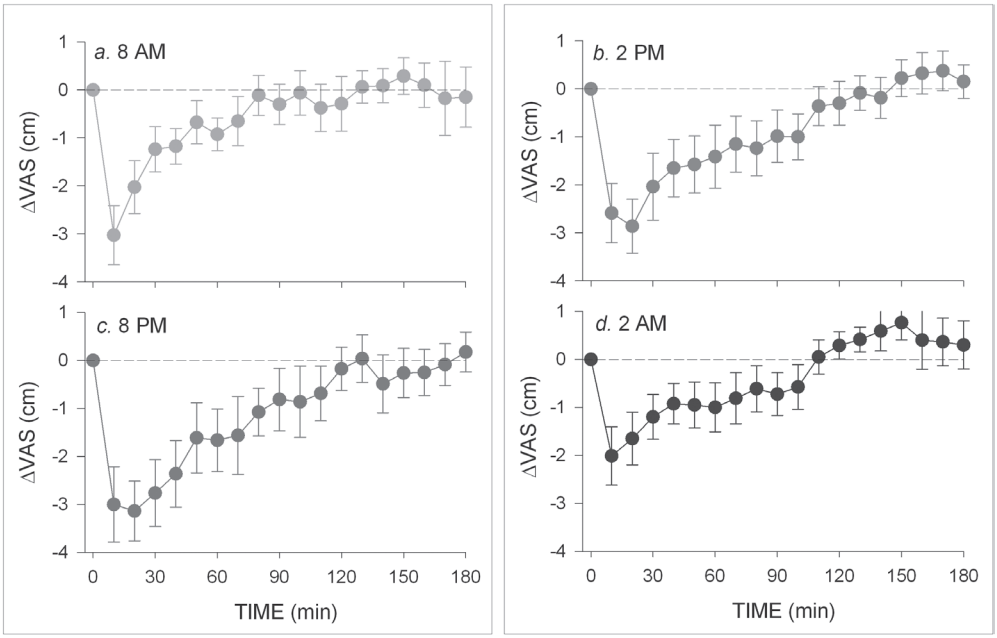

Figure 1. Effect of fentanyl on heat pain scores in two groups of subjects. One group received intravenous 2.1 $\mu g.kg^{-1}$ fentanyl at 8 AM and 8PM (**A** and **C**), the other group at 2 PM and 2 AM (**B** and **C**). Values are mean ± SD.

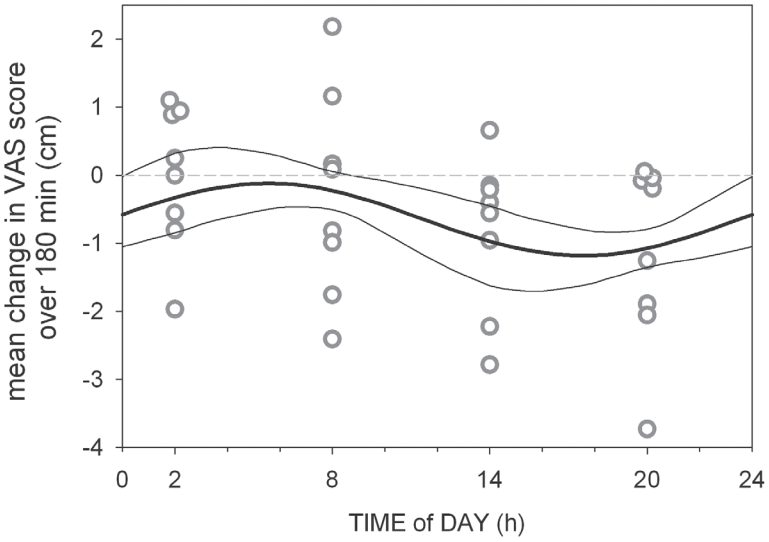

Figure 2. Mean pain scores after injection of 2.1 $\mu g.kg^{-1}$ fentanyl observed at 2 AM, 8AM, 2 PM and 8 PM.

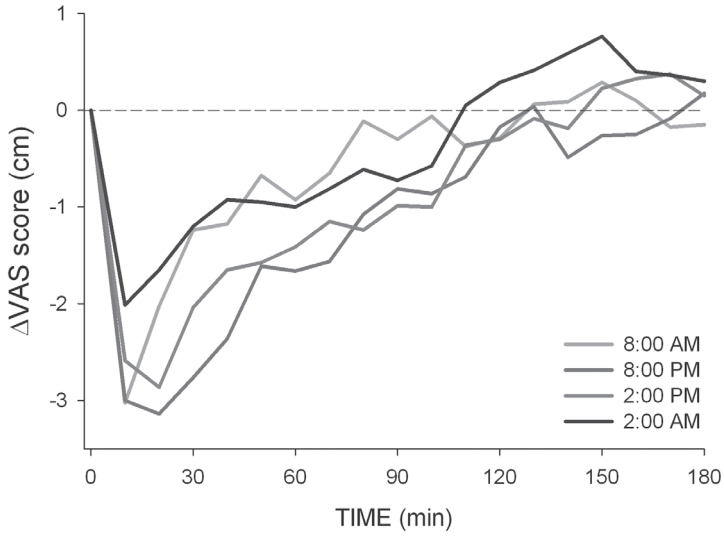

Figure 3. Data fit of analgesic effect from 2.1 $\mu g.kg^{-1}$ iv fentanyl versus time of day at which the drug was injected. Analgesic effect is defined as the mean change in VAS over the 180 min study period. Each circle represents the analgesic effect of one subject. The fit is a sinusoidal curve (thick continuous line) ± 95% confidence interval (thin continuous lines). The broken line denotes a separation between mean analgesic responses (data below the broken line) and hyperalgesic responses (above the broken line).

The negative value of the offset indicates that on average at all times an analgesic response occurred. An amplitude of 0.65 means that the average VAS varied by 1.3 cm over time (recalculation for just the first 90 min of the experiment would yield a variation in VAS of 2 cm; Note that these variations are model predictions). The value of φ of 27 degrees indicates that at midnight (0 h in figure 3) the sinus was shifted by 27 degrees. The frequency value f was fixed to 1 as we assumed that the sinus occurred once every 24 h. Fentanyl was most analgesic in the late afternoon and early evening hours (between 2 PM and 8 PM, minimum of the sinus occurred at 5 AM), while it was least analgesic in the early morning hours (from 2 AM to 8 AM, maximum of the sinus occurred at 3 PM).
Side effects showed much less of a variation over time than analgesia with no differences among observations within and between groups (anova: $P > 0.05$; table 1). A significant sinus could not be demonstrated for end-tidal PCO_2 or sedation.

Table 1. Baseline parameter values and 3-hour area-under-the-time-effect curve for end-tidal CO_2 and sedation

	8 AM – 8 PM	**2 AM – 2 PM**
Baseline values		
Temperature of thermode (°C)	48.5 ± 0.8 – 48.6 ± 0.8	48.2 ± 1.5 – 48.7 ± 1.4
Baseline pain VAS (cm)	7.8 ± 0.4 – 7.7 ± 0.4	6.6 ± 0.9 – 6.8 ± 0.7
Baseline CO_2 (vol. %)	4.7 ± 0.6 – 5.1 ± 0.5	4.7 ± 0.8 – 4.6 ± 0.5
Baseline sedation NRS (cm)	3.1 ± 0.7 – 1.9 ± 0.8	2.0 ± 0.4 – 1.0 ± 0.3
3 h AUEC's		
CO_2 (time.vol. %)	47 ± 22 – 41 ± 21	33 ± 40 – 48 ± 14
Sedation (time*cm)	125 ± 131 – 198 ± 153	188 ± 110 – 272 ± 190

No significant differences in parameter values were obtained among the study times (analysis of variance: $P > 0.05$)

6.4 DISCUSSION

We observed a circadian sinusoidal rhythm in the analgesic effect of fentanyl. Variations were observed for peak analgesic effect, duration of effect and the occurrence of hyperalgesia. When using AEC as end-point, we observed a peak in pain relief late in the afternoon (5:30 PM) and a trough in the early morning hours (5:30 AM). The difference between the peak and trough in pain relief corresponds to a difference in VAS of 1.3 to 2 cm. This indicates that the magnitude of the diurnal variation of fentanyl analgesia is significant albeit relatively small with increased sensitivity to fentanyl in the late afternoon and evening hours (1 to11 PM).

6.4.1. OPIOID EFFECT ON THE CIRCADIAN RHYTHM

In mammals the suprachiasmatic nucleus (SCN) in the hypothalamus is the site that controls circadian behavioral rhythmicity (*i.e.*, the master clock).[7,8] The SCN is

synchronized by external stimuli of which the light/dark cycle is the most important (the retina is directly linked to the SCN via the retinohypothalamic tract). Other synchronizers include locomotor activity, drugs (*e.g.*, benzodiazepines, opioids, serotonin agonists) and social interaction. The SCN controls many cyclic events in the mammalian body including the synthesis and release of hormones such as melatonin and cortisol and body temperature. Generation of rhythmicity in the SCN is genetically determined and based on feedback loop that involves several genes, including *Per1*, *Per2* and Clock.[7,8] The SCN and its afferent and efferent pathways contain various neurotransmitters including neuropeptide Y, γ-amino butyric acid (GABA) and enkephalins. The role of enkephalins in the circadian system has received increasing attention as δ-opioid receptors were identified in the hamster SCN and the μ-opioid receptor agonist fentanyl induces a phase shift in the circadian rhythm of hamsters independent of any behavioral effects of the opioid.[9,10] We showed previously that fentanyl modifies the circadian pacemaker possibly via direct effects on SCN electrical activity and regulation of *Per* genes.[9] This suggests that pathways regulating the circadian clock intersect directly or indirectly with pathways that express opioid receptors. Our current study, in which a diurnal variation in fentanyl's analgesic behavior is observed (*i.e.*, an effect opposite to fentanyl's influence on the clock), similarly suggests involvement of the opioid system in the circadian rhythm.

We refrained from measuring plasma fentanyl concentrations in our observational study. We argued that frequent blood sampling could interfere with the subjects rating of heat pain possibly causing stress-induced analgesia that encompasses strong circadian variations.[11] Consequently, the variation in fentanyl's effect may be due to a true increase in the opioid's antinociceptive efficacy (a pharmacodynamic effect), as suggested above, but we cannot exclude a diurnal variation in fentanyl's pharmacokinetics. An increase in plasma fentanyl concentrations in the late afternoon and early evening may well explain our findings. Variations in plasma morphine concentrations following oral administration in patients with cancer pain have been observed due to variations in absorption and/or changes in the volume of distribution over a 24 h period.[12] Similarly, intramuscular meperidine injections in patients with sickle cell anemia were associated with circadian changes in drug disposition and elimination over the day.[2] In contrast, oral codeine and tramadol given to healthy volunteers in the morning or evening did not show any differences in pharmacokinetics.[4] Similarly, and of importance to our study, in two separate studies in volunteers receiving intravenous fentanyl, the plasma fentanyl concentration-time profiles were independent of the time of infusion.[13] This then suggests that our findings are related to a circadian effect on fentanyl's pharmacodynamics and not to its pharmacokinetics.

6.4.2 CIRCADIAN VARIATIONS IN PAIN RESPONSE

Several animal studies showed that the response to noxious stimuli is not constant over a 24 h period.[1,2,11,14] The results of human experimental and clinical studies are less clear

with some studies finding no difference in pain over time, while other found more pain in the morning or evening.[1-3,15,16] Experimental pain studies indicate that variations in pain sensitivity depend on the tissue tested and the nociceptive assay employed.[1-3,15,16] Using a similar thermode as we did, Strian *et al*.[15] did observe a variation in pain threshold values to warm and cold stimuli but these variations were small and had no consistent pattern among subjects. Our study was not designed or powered to study variations in pain sensitivity and, as expected, we did not observe significant differences in temperature to induce VAS > 5 cm. However, at this point we cannot exclude some effect of variations in pain sensitivity on the antinociceptive responses that we observed with less pain reporting between 1 and 11 PM (and hence a greater analgesic response at these times). Indeed, skin sensitivity to heat is minimal at 6 PM and maximal at 6 AM and also painful stimulation of the nasal mucosa with carbon dioxide is increased during evening test sessions.[3,17] Further studies are needed to investigate the complex interaction between variations in pain sensitivity and opioid treatment. An important question in this respect is, for example, whether the pain and analgesic rhythms display antagonistic or synergistic interactions.

6.4.3 MECHANISMS OF OPIOID CIRCADIAN RHYTHM

The mechanism though which the circadian rhythm affects opioid analgesic efficacy remains unknown. Variations in hormones (*e.g*., cortisol, melatonin) and endogenous opioid peptides (meta-enkephalin and β-endorphins) could play an important role interacting with the nociceptive pathways and opioid system.[18-20] For example, the analgesic effect of melatonin is more pronounced at night.[21] An interesting observation in mice is that μ-opioid receptor expression displays a 24-h rhythm.[22] Down regulation of the brain μ-opioid receptor was associated with a decrease in morphine analgesia. Extrapolation of these animal data to ours in humans then suggests that during the late evening, morning and early afternoon, human μ-opioid receptors are down regulated via a direct or indirect (e.g. hormonal) influence of the SCN. Our finding of enhanced analgesic fentanyl efficacy from 1 to 11 PM is in agreement with other human studies showing similar patterns of opioid effect. Non-lethal opioid overdose (*i.e*., increased opioid sensitivity causing respiratory depression) shows a significant peak in the afternoon and early evening, an effect that was independent of the opioid plasma concentrations.[23] Oral codeine and tramadol display greater analgesic sensitivity when administered in the early evening.[4]

6.4.4 HYPERALGESIA

A somewhat surprising observation in our study was the occurrence of a moderate hyperalgesic response (pain sensitivity greater than baseline) following analgesia in subjects receiving fentanyl at 2 AM (figure 1). This phenomenon was outspoken in 5 subjects tested at 2 AM and occurred on 11 occasions in the whole study (figure 3). Hyperalgesia in response to opioids has been observed in various species, including

humans. Recent data indicate that opioid-induced hyperalgesia is not related to activation of opioid receptors but possibly due to activation of N-methyl-D-aspartic acid receptors within pain pathways.[24,25] Animal studies showed that hyperalgesia induced by opioid-receptor blockade by naloxone (*i.e.*, a non opioid receptor phenomenon) follows a diurnal rhythm.[26] This then suggests that our results may have been influence by three separate rhythms: an inherent pain rhythm, a fentanyl analgesic and anti-analgesic rhythm.

6.4.5 CRITIQUE OF METHODS

It is possible that the observed rhythm is entirely due to the use of two distinct subject groups, one of which was studied at 8 AM and 8 PM, the other at 2 PM and 2 AM. This could, for example, occur when the two groups would differ in their AEC's without a within-group difference between measurement points (for example: [AEC(group 1) at 2 PM = 2 AM] > [AEC(group2) at 8 AM = 8 PM]). However, this was not the case (figures 1 and 2). In both groups the data collected in the morning hours (2 AM or 8 AM) displayed a peak effect and AEC of lesser magnitude than the data collected in the afternoon or early evening hours (2 PM or 8 PM). This suggests that the observed rhythm was inherently present in the two study groups and not related to the design of the study.

We modeled the data with a symmetrical sinusoid function. This function was significantly better than a linear function. We did assess also non-symmetrical sinusoid functions by allowing the four parts of the sinusoid to vary independently in amplitude (with factor FAC). However, no significant improvements in minimum objective function were observed in comparison to FAC values of 1. Furthermore, assessing the residuals of the symmetrical sinusoid functions showed the absence of any bias (means residuals per test period not different from zero). This indicates that the sinusoid chosen adequately described the data.

We subtracted the baseline pain score from the VAS-time data to allow objective assessment of the change in VAS over time (AEC). This was possible in our data set as we observed little variation in the baseline VAS (*i.e.*, predrug) score. We cannot exclude, however, that some error in baseline values may erroneously propagate to the estimates of the model parameters. However, in our analysis, the error only propagates to the inter-individual variability of the model parameter offset. We tested the variance in offset and observed that it was not different from zero, suggesting that subtraction of baseline pain scores did not affect our study outcome. In some studies the analysis of circadian effects is sensitive to 'edge effects' or the moment in time defined as the start of day or start of analysis (this is often related to the use of a smoothing function).[27] We chose midnight as starting point of our analysis. Our NONMEM analysis of the data with a non-smoothed sinusoid does not have any edge effects.

Recent studies on chronopharmacology of labor analgesia with intrathecal bupivacaine indicate that one has to be careful with the interpretation of rhythmic patterns in the duration of analgesia.[27] This concerns patient studies in which daily routines (external

rhythms such nursing and anesthesia provider shifts) produce artifacts (suggesting a biological rhythm in intrathecal analgesia duration) that have little to do with biological rhythms.[27,28] We were aware of these pitfalls and designed our study to prevent influences from external rhythms. However, despite our efforts we cannot exclude some albeit small effect from external sources on our study outcome.

6.5 CONCLUSIONS

We observed a circadian rhythm in the analgesic effect of fentanyl in human volunteers using an experimental heat pain model. Our data indicate an increase in analgesic efficacy in the late afternoon and early evening hours. We argue that the most probable cause for our findings is chrono-pharmacodynamic effect regulated by the circadian clock in the hypothalamus. This may be a direct effect through shared pathways of the circadian system and the opioid system or an indirect effect via diurnal variations in hormones or endogenous opioid peptides that rhythmically change the pain response and/or analgesic response to fentanyl.

REFERENCES

1. Labrecque G, Vanier M-C. Biological rhythms in pain and in the effects of opioid analgesics. *Pharm Tehr* 1995; 129-47.
2. Chassard D. Bruguerolle B. Chronobiology and anesthesia. *Anesthesiology* 2004 100: 413-27.
3. Dahan A. Circadian influences, low-dose isoflurane, and the ventilatory response to hypoxia. *Anesthesiology* 2005; 103: 2-7-8.
4. Hummel T, Kratesch H-G, Lötsch J, Hepper M, Liefhold J, Kobal G. Analgesic effects of dihydrocodeine and tramadol when administered either in the morning or evening. *Chronopharm Int* 1995; 12: 62-72.
5. Olofsen E, Romberg R, Bijl H, Mooren R, Engbers F, Kest B, Dahan A: Alfentanil and placebo analgesia: no sex differences detected in models of experimental pain. *Anesthesiology* 2005; 103: 130-9.
6. Beal BL, Sheiner LB, Boeckman AJ: NONMEM User's Guide. Icon development Solutions, Ellicott City, Maryland, USA. 1989-2006.
7. Hastings M. The brain, circadian rhythms, and clock genes. *Br Med J* 1998; 317: 1704-7.
8. Wager-Smith K, Kay SA. Circadian rhythm genetics: from flies to mice to humans. *Nat Genet* 2000; 26: 23-7.
9. Vansteensel MJ, Magnone MC, van Oosterhout F, Baeriswyl S, Albrecht U, Albus H, Meijer JH. The opioid fentanyl affects light input, electrical activity and Per gene expression in the hamster suprachiasmatic nuclei. *Eur J Neurosci* 2005; 21: 2958-66.
10. Meijer JH, Ruijs ACJ, Albus H, van de Geest B, Duindam H, Zwinderman AH, Dahan A. Fentanyl, a m-opioid receptor agonist, phase shifts the hamster circadian pacemaker. *Brain Res* 2000; 868: 135–40.
11. Puglisi-Allegra S, Castellano C, Oliverio A. Circadian variations in stress-induced analgesia. *Brain Res* 1982; 252: 373-6.
12. Gourlay GK, Plummer JL, Cherry DA. Chronopharmacokinetic variability in plasma morphine concentrations following oral doses of morphine solution. *Pain* 1995; 61: 375-81.
13. Gupta SK, Southam MA, Hwang SS. Evaluation of diurnal variation in fentanyl clearance. *J Clin Pharmacol* 1995; 35:159-62.
14. Kavaliers M, Hirst M. Daily rhythms of analgesia in mice: Effects of age and photoperiod. *Brain Res* 1983; 279: 387-93.
15. Strian F. Lautenbacher S. Galfe G. Holzl R.

Diurnal variations in pain perception and thermal sensitivity. *Pain* 1989; 36: 125-31.

16. Bruera E, Macmillan M, Kuehn N, Miller MJ. Circadian distribution of extra doses of narcotic analgesics in patients with cancer pain: A preliminary report. *Pain* 1992; 49: 311-4.
17. Procacci P, Corte MD, Zoppi M, Maresca M. Rhythmic changes of the cutaneous pain threshold in man: A general review. *Chronobiol* 1974; 1: 77-96.
18. Hamra JG, Kamerling SG, Wolfsheimer KJ, Bagwell CA. Diurnal variation in plasma ir-beta-endorphine levels and experimental pain thresholds in the horse. *Life Sci* 1993; 53: 121-9.
19. Wesche DL, Frederickson RCA. The role of the pituitary in the durnal variation in tolerance to painful stimuli and brain enkephalin levels. *Life Sci* 1981; 29: 2199-205.
20. Hindmarsch KW, Tan L, Sankaran K, Laxdal VA. Diurnal rhythms of cortisol, ACTH, and β-endorphin levels in neonates and aduts. *West J Med* 1989; 151: 152-6.
21. Ebadi M, Govitrapong P, Phansuwan-Pujito P, Nelson F, Reiter RJ. Pineal opioid receptors and analgesic action of melatonin. *J Pneal Res* 1998; 24: 193-200.
22. Yoshida M. Ohdo S. Takane H. Tomiyoshi Y. Matsuo A. Yukawa E. Higuchi S
Chronopharmacology of analgesic effect and its tolerance induced by morphine in mice. *J Pharmacol Exp Ther* 2003; 305:1200-5.
23. Gallerani M, Manfredi R, Dal Monte D, Calo G, Brunaldi V, Simonato M. Circadian differences in the individual sensitivity to opiate overdose. *Crit Care Med* 2001; 29: 96-101.
24. van Dorp ELA, Kest B, Kowalczyk WJ, Morariu AM, Waxman AR, Arout CA, Dahan A, Sarton EY. Morphine-6-glucuronide rapidly increases pain sensitivity independently of opioid receptor activity in mice and humans. *Anesthesiology* 2009; 110:1356-63.
25. Waxman AR, Arout C, Caldwell M, Dahan A, Kest B. Acute and chronic fentanyl administration causes hyperalgesia independently of opioid receptor activity in mice. *Neurosci Lett* 2009; 462: 68-72.
26. Fredrickson R. Hyperalgesia induced by naloxone follows diurnal rhythm in responsivity to painful stimuli. *Science* 1977; 198: 756-8.
27. Shafer SL, Lemmer B, Boselli E, Boiste F, Bouvet L, Allaouchiche B, Chassard D. Pitfalls in chronobiology: Intrathecal bupivacaine for labor analgesia. *Anesth Analg* 2010, in press.
28. Scavone BM, McCarthy RJ, Wong CA, Sullivan JT. The influence of time of day of administration on duration of labor analgesia. *Anesth Analg* 2010, in press.