Anesthesiology
74:166–171, 1991

Acute Tolerance in Morphine Analgesia: Continuous Infusion and Single Injection in Rats

Igor Kissin, M.D., Ph.D., Pamela T. Brown, B.S.,† C. Andrew Robinson, Ph.D.,‡ Edwin L. Bradley, Jr., Ph.D.§*

This study aimed to determine whether the decline of the analgesic effect of morphine with a continuous infusion or that after a single injection correlates with the changes in brain concentration of morphine. The analgesic effect of morphine and its brain and serum concentrations were determined with a continuous 8-h infusion at a constant rate and after a single subcutaneous injection of the agent. The analgesic effect was determined by measuring the threshold of motor response to noxious stimulation. Brain and serum concentrations of morphine were detected by radioimmunoassay with the use of ^{125}I-labeled morphine. With the constant-rate (4 mg · kg^{-1} · h^{-1}, intravenous) morphine infusion, the peak of analgesia could not be maintained: the increase in the pain threshold at 2 h was 1,003 g and at 8 h was 286 g (a decrease in analgesia by 72%, $P < 0.0002$). At the same time, the brain morphine concentration tended to increase, to 278 ng/g at 2 h and 329 ng/g at 8 h. After the single morphine injection (6 mg/kg, subcutaneous), recovery from analgesia occurred at a much faster rate than did the decrease in morphine brain concentration; the decrease in pain threshold was 79% at 90 *vs.* 30 min after the injection ($P < 0.0001$), and the corresponding decrease in brain concentration was 28% (NS). The absence of correlation between analgesia and morphine brain concentration both with the constant-rate morphine infusion and after the single injection suggests the development of acute tolerance, which is pharmacodynamic in nature. (Key words: Analgesics, opioid: morphine. Pharmacokinetics: drugs, brain concentration; infusion. Potency, analgesic: tolerance.)

MORPHINE CONCENTRATION in the cerebrospinal fluid (CSF) of patients was found by Nordberg *et al.*[1] to reach its peak 3 h after intramuscular injection of the drug (10 mg) and not to decrease appreciably for the next 2 h. The authors did not determine the analgesia, but according to clinical evidence, the analgesic effect of 10 mg morphine disappears 5 h after its administration.[2] The results obtained by Nordberg *et al.* correlate well with data indicating that the concentration of morphine in the brain tissue of dogs does not significantly decrease 5–8 h after intravenous or subcutaneous injection of the drug.[3,4] Hug *et al.*[5] have found in dogs that the elimination of morphine from CSF was of much longer duration than that from plasma. Studies in which a constant rate of morphine was given by intravenous infusion for 8 h demonstrated a profound decrease of morphine analgesia with time both in dogs[6] and in rats.[7]

Although the morphine concentration was not examined in the infusion studies, it was suggested that development of acute tolerance explains the observed phenomenon. The slower decline of morphine brain concentration as compared to recovery from the analgesic effect may indicate that even a single injection of morphine leads to the development of acute tolerance, shortening the duration of analgesia induced by this injection.

The purpose of the current study was to determine whether the decline of the analgesic effect of morphine with a continuous infusion or after a single injection correlates with the changes in brain concentration of morphine.

Materials and Methods

Experiments were performed on male Sprague-Dawley rats weighing 225–275 g. The protocol for this study was approved by the Institutional Panel on Laboratory Animal Care. Analgesia was determined by measuring the threshold of motor response to increasing noxious pressure applied to the tail[8] with the use of an Analgesy-Meter (Ugo Basile, Milan, Italy). The rat's tail was positioned on a Teflon platform, and the pressure plate (0.7-mm edge) attached to this device was placed 4.5 cm from the tip of the tail while the rat was held in the experimenter's hand. Pressure was increased at a constant rate (cut-off pressure of 2.75 kg) until the animal made an attempt to escape. The pressure at that moment was recorded, and the mean of three consecutive measurements was taken as the reaction threshold.

Two series of experiments were performed, one (series 1) with infusion of morphine for 8 h at a constant rate (Harvard Apparatus Compact Infusion Pump, model 975), and another (series 2) with a single injection of the drug. In series 1, a catheter for the drug infusion was chronically implanted into the jugular vein, and its free end was exteriorized through the skin at the back of the neck. The surgical procedure for implantation was performed under pentobarbital anesthesia (50 mg · kg^{-1}, intraperitoneal) several days before the experiment.

Series 1 consisted of six groups of experiments: group

* Professor of Anesthesiology, Department of Anesthesiology.

† Research Assistant, Department of Anesthesiology.

‡ Associate Professor of Pathology, Director of Special Chemistry/Toxicology Laboratory.

§ Professor of Biostatistics, Department of Biostatistics and Biomathematics.

Received from the Department of Anesthesiology, University of Alabama School of Medicine, University of Alabama at Birmingham, Birmingham, Alabama. Accepted for publication September 21, 1990.

Address correspondence to Dr. Kissin: Department of Anesthesiology, University of Alabama School of Medicine, University of Alabama at Birmingham University Station, Birmingham, Alabama 35294.

A (pilot group, n = 7) received an infusion of morphine at a rate of 4 $mg \cdot kg^{-1} \cdot h^{-1}$ (3 $ml \cdot kg^{-1} \cdot h^{-1}$) for 8 h and had seven determinations of pain threshold, at the following time intervals: baseline and 0.5, 1, 2, 4, 6, and 8 h. (The rate of infusion was selected to provide maximal individual increases in the pain threshold that do not exceed the cut-off pressure.) Group B (n = 5) received an infusion of normal saline 3 $ml \cdot kg^{-1} \cdot h^{-1}$ and had seven determinations of pain threshold at the above time intervals. Group C (n = 8) received an infusion of morphine at the rate of 4 $mg \cdot kg^{-1} \cdot h^{-1}$ and had only two determinations of pain threshold, at 2 and 8 h.

In the rest of the experiments (groups D, E, and F) the rats were killed (by decapitation) randomly at different time intervals from the beginning of infusion of morphine (4 $mg \cdot kg^{-1} \cdot h^{-1}$), and brain and blood samples were taken for the determination of morphine concentration by an investigator blinded to the treatment. In group D (n = 8), the animals were decapitated 0.5 h after the beginning of the infusion, after two determinations of pain threshold (baseline and immediately before the decapitation) had been made. In group E (n = 8), animals were killed 2 h after the beginning of infusion, after three determinations of pain threshold (baseline, 0.5 h, and immediately before decapitation) had been made. In group F (n = 8), animals were decapitated 8 h after the beginning of infusion, after four determinations of pain threshold (baseline, 0.5 and 2 h, and immediately before decapitation) had been made.

In series 2, the effect of a single subcutaneous injection of morphine was studied. The series consisted of six groups of animals. Group A (pilot group, n = 7) received a subcutaneous injection of morphine 6 mg/kg and had five determinations of pain threshold, at the following time intervals: baseline, and 5, 30, 60, and 120 min. Group B (n = 5) received a subcutaneous injection of normal saline and had five determinations of pain threshold at the same time intervals as in group A. In groups C, D, E, and F, animals (n = 8 for each group) were decapitated randomly at different time intervals after the injection of morphine in a subcutaneous dose of 6 mg/kg, and the brain and blood samples were taken for the determination of morphine concentrations. In group C, animals were decapitated 5 min after the injection of morphine, after two determinations of pain threshold (baseline and immediately before the decapitation) had been made. In groups D, E, and F, animals were decapitated at 30, 60, and 90 min, after three (baseline and 5, and 30 min), four (baseline, and 5, 20, and 60 min) or five (baseline, and 5, 30, 60, and 90 min) pain threshold measurements, respectively, had been made.

After decapitation, the mixed blood sample was obtained, allowed to clot, and centrifuged, and the serum was refrigerated. The brain was excised, freed of blood vessels and choroid plexus as much as possible, weighed, and also refrigerated. Brain and serum concentrations of morphine were determined by a radioimmunoassay with the use of ^{125}I-labeled morphine (Kit Abuscreen®, Roche Diagnostic Systems, Hoffman-LaRoche, Inc., Nutley, NJ). The whole brain was homogenized in 0.1 M sodium phosphate buffer, *p*H 8.9, at 1 g brain per 2 ml buffer. The blood sample was diluted 1:10 with drug-free serum. The assay for morphine was performed with the use of a procedure described by Edwards *et al.*[9] To prevent the antibody cross-reactivity with morphine-3-glucuronide, which is as high as 38%, this procedure uses a chloroform extraction of both standards and unknowns. The extraction completely isolates morphine from morphine-3-glucuronide.

For calculations, standard curves were prepared by plotting percent bound ^{125}I-morphine *versus* morphine concentration. A best-fit curve for this relationship was obtained with the RIA Data Reduction computer program (American Society of Clinical Pathologists). Sample concentrations were obtained by interpolating from the standard curve. The sensitivity of the radioimmunoassay for morphine is 10 ng/ml and the variability ±10%.

Morphine sulfate used in this study was purchased from Robins Co. (Cherry Hill, NJ). Doses of morphine were expressed in terms of the sulfate salt.

Comparisons among the times after treatment were made with a one-way analysis of variance.[10] Paired tests between any two time intervals within a treatment were carried out with Fisher's protected least significant difference test.[10] Differences were considered statistically significant if P was <0.05.

Results

Results of the constant-rate infusion experiments (groups A and B) are presented in figure 1. Morphine infusion at the rate of 4 $mg \cdot kg^{-1} \cdot h^{-1}$ caused an increase in pain threshold that reached its peak at 2 h. After this the analgesia began to decline, and at 8 h the pain threshold increase was only one fourth of the peak value and no different from control animals receiving saline. The infusion of saline for 8 h (group B) did not cause any significant changes in pain threshold. In group C, pain threshold was measured only twice, at 2 and 8 h after the beginning of morphine infusion (fig. 2). The figure demonstrates that, despite the constant rate of morphine administration, the decrease in pain threshold was the same as with the seven pain threshold determinations in group A.

Figure 3 represents the results obtained in groups D, E, and F; it compares changes in pain threshold with morphine brain concentrations at 0.5, 2, and 8 h. The most important finding was a tendency for increase in the morphine brain concentration at 8 h compared to that at 2 h

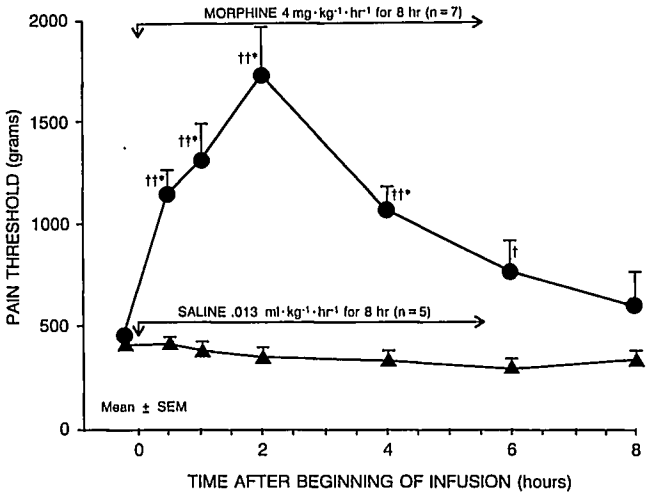

FIG. 1. The effect of intravenous morphine infusion at a constant rate on the pain threshold. *$P < 0.002$ from baseline; †$P < 0.02$ from saline; ††$P < 0.001$ from saline.

(18% increase, nonsignificant [NS]) despite a profound decrease of analgesia during the same time interval (−72%, $P < 0.0002$). The increase in pain threshold at 8 h was less than that at 0.5 h (286 *vs.* 556 g, $P < 0.002$), although the morphine brain concentration at 8 h was more than twice as great as that at 0.5 h (329 *vs.* 134 ng/g, $P < 0.0001$). The relationship between brain and serum morphine concentrations is presented in figure 4. The serum concentration was many times higher than the brain concentration, especially at the beginning of the infusion (serum/brain ratio = 18.4 at 0.5 h and 10.5 at 8 h). The morphine serum and brain concentrations at 8 h were not significantly different from those at 2 h; in the brain concentration there was a tendency for an increase at 8 h.

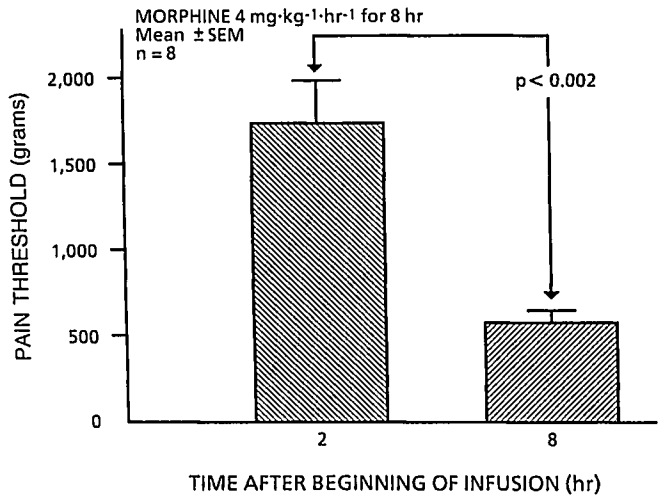

FIG. 2. Acute tolerance to the analgesic effect of morphine, demonstrated with two measurements of pain threshold.

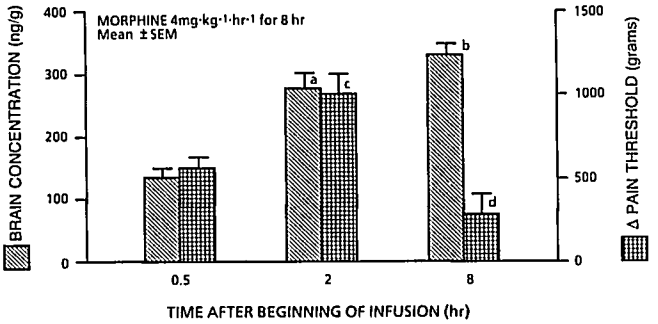

FIG. 3. The correlation between changes in the pain threshold and brain morphine concentration with constant rate morphine infusion. Columns represent brain morphine concentrations and changes in pain threshold at 0.5, 2, and 8 h after the beginning of the infusion (three groups of rats, eight animals in each group). a = $P < 0.0001$ from 0.5 h; b = NS from 2 h; c = $P < 0.01$ from 0.5 h; d = $P < 0.0002$ from 2 h.

The effect of a single morphine injection on pain threshold (series 2, groups A and B) is presented in figure 5. The highest pain threshold was at 30 min after the injection. Figure 6 compares the changes in pain threshold with the morphine brain concentrations (groups C, D, and E). Two discrepancies in the relationship between pain threshold and morphine brain concentration can be seen in this figure. First, the recovery of pain threshold after morphine injection develops much faster than does the decrease in morphine brain concentration: there was a profound decrease in analgesia at 90 min compared to that at 30 min (−79%, $P < 0.0001$) at a time when the brain concentration had only slightly decreased (−28%,

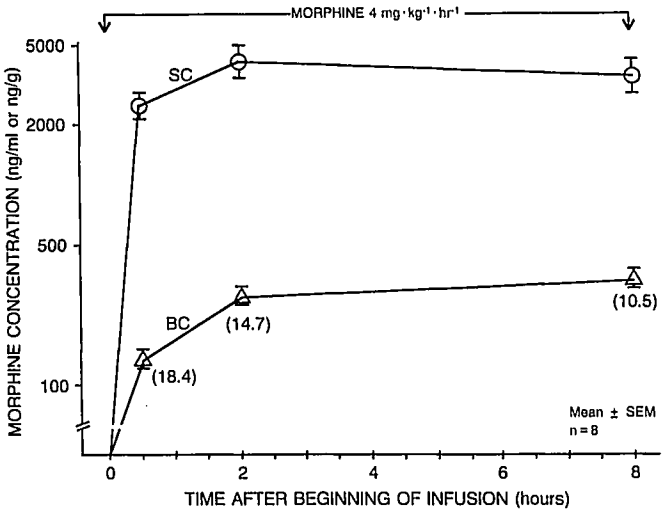

FIG. 4. The relationship between brain and serum morphine concentrations with the constant rate drug infusion. Morphine concentration is plotted on a logarithmic scale. The ratios of serum (SC) to brain (BC) morphine concentration are in parentheses.

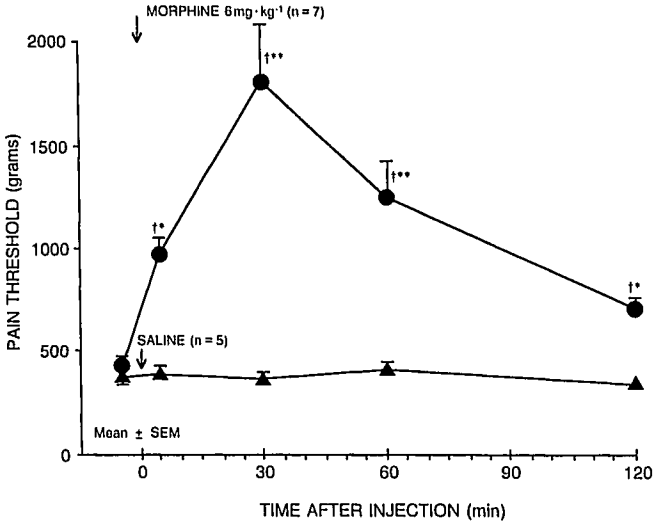

FIG. 5. The effect of a single injection of morphine on the pain threshold. *$P < 0.05$ from baseline; **$P < 0.0001$ from baseline; †$P < 0.005$ from saline.

NS). Second, the increase in pain threshold at 90 min was less than that at 5 min (321 *vs.* 602 g pressure, $P < 0.001$), although the morphine brain concentration tended to increase (207 ng/g at 90 min *vs.* 160 ng/g at 5 min, NS).

Figure 7 represents the relationship between brain and serum morphine concentrations after a subcutaneous injection of morphine. Five minutes after the injection of morphine, the serum concentration was 14-fold higher than that in the brain. Then, the serum/brain ratio rapidly began to decrease, and the blood and brain morphine decrement curves intersected at a time interval between 60 and 90 min. A rapid decrease in the morphine serum concentration was in sharp contrast to the insignificant decline of the morphine brain concentration during the 30–90-min time interval.

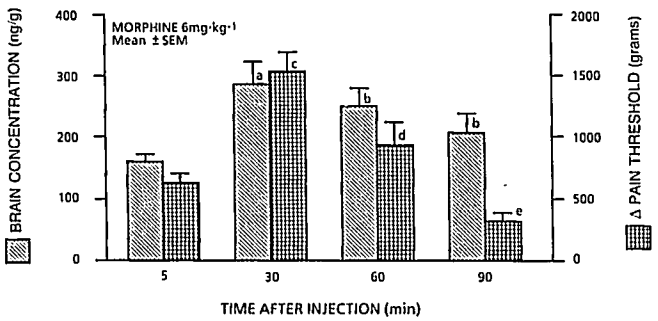

FIG. 6. The correlation between changes in the pain threshold and morphine brain concentration after a single morphine injection. Columns represent brain morphine concentrations and changes in pain threshold determined at four time intervals (four groups of rats, eight animals in each group). a = $P < 0.005$ from 5 min; b = NS from 30 min; c = $P < 0.0002$ from 5 min; d = $P < 0.01$ from 30 min; e = $P < 0.01$ from 60 min.

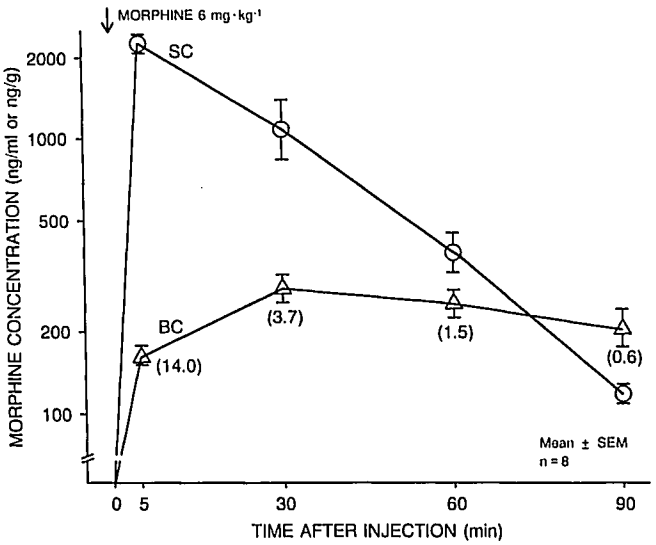

FIG. 7. The relationship between brain and serum morphine concentrations after a single morphine injection. Morphine concentration is plotted on a logarithmic scale. The ratios of serum (SC) to brain (BC) morphine concentration are in parentheses.

Discussion

Our experiments demonstrated that during the 8-h constant-rate infusion of morphine, the analgesic effect was not maintained, and the degree of analgesia began to decrease at 4 h and was reduced by three fourths at 8 h. The extent of this reduction was independent of the number of pain threshold determinations (as is seen by comparing results of seven and two determinations; figs. 1 and 2, respectively). This finding corresponds well to the results reported previously.[6,7] Morphine brain concentration between the peak of analgesia and its maximal decline during the course of infusion did not decrease, indicating that the acute tolerance developed during the morphine infusion does not result from a decrease in the brain concentration of the drug.

The results of the single-injection experiments revealed a slower decrease in morphine brain concentration as compared to recovery from analgesia: the decrease in morphine brain concentration was 28% (NS) and the decrease in analgesia 79% ($P < 0.0001$), at 90 min after the injection compared to 30 min. In both single-injection and continuous-infusion experiments, the ratio of analgesia to morphine brain concentration during the rise of analgesia was much higher than that during the decline of analgesia. At 8 h with infusion and at 90 min with single injection, the analgesia was approximately half of the initial values (initial values taken at 0.5 h and 5 min, respectively), at a time when the morphine brain concentration was significantly increased when compared to initial values. Absence of correlation between analgesia and morphine brain concentration during recovery after the

drug injection can be explained by the development of acute tolerance that accelerates the process of recovery.

The comparison of morphine brain and serum concentrations demonstrated that in the beginning of morphine infusion, the serum concentration was 18-fold higher, with a small decrease in this difference at the end of infusion. With single-injection experiments, the morphine serum concentration was 14-fold higher than the brain concentration at 5 min. However, at 90 min, the above relationship was reversed: the morphine serum concentration was lower than the brain concentration. The intersection of serum and brain morphine decrement curves, and the very small decline of the brain curve, correspond well to the results by Nishitateno *et al.*,[4] who reported an insignificant decline in brain morphine concentration for 6 h after its intravenous injection at a time when the serum morphine concentration was reduced very rapidly. In their study, the half-life of morphine in the brain was 5 times longer than that in the serum. Mule and Woods demonstrated the slower decrease of morphine concentration in the CSF as compared to plasma, including the crossover of the concentrations.[3] Hug *et al.*[5] found that elimination of morphine from the CSF was much longer than from plasma.

In contrast to the above data, Dahlström and Paalzow[11] could not find any difference in the morphine plasma/brain ratio during the 20–240-min interval after intravenous morphine injection. They also reported[12] that morphine brain concentration decreases faster than does morphine-induced analgesia, a finding that contradicts the results obtained in our study and in several other studies.[4,13]

The major mechanism of morphine biotransformation is its conversion to morphine-3-glucuronide in the liver. This mechanism is so active that by 10–20 min after intravenous injection of morphine, the concentration of morphine metabolites in plasma exceeds that of unchanged morphine in dogs.[5] The penetration of glucuronides through the blood–brain barrier is restricted, and there is no evidence for the formation of morphine metabolites in the central nervous system. However, the ratio of morphine metabolites to morphine in the brain is constantly increasing after morphine administration. Morphine-3-glucuronide has only one tenth of the morphine analgesic potency.[14] Therefore, if the method of radioimmunoassay for morphine has a high degree of cross-reactivity with morphine-3-glucuronide, the morphine brain concentration values may be overestimated with the increase in the time after injection. The cross-reactivity with morphine-3-glucuronide in our experiments was eliminated through the use of a specific extraction procedure.[9] This was clearly confirmed by the data presented in figure 7: during the 5–90-min period, morphine serum concentration decreased from 2,000 to 120 ng/ml. Hug *et al.*[5] reported that during the same time period, the plasma level of conjugated morphine had increased to the degree that at 90 min it exceeded the concentration of unchanged morphine by 10-fold. These data indicate that a 20-fold decrease in morphine serum concentration in our experiments would not be possible unless the extraction procedure used did not eliminate cross-reactivity between morphine and morphine-3-glucuronide.

Morphine is also converted to morphine-6-glucuronide. This metabolite of morphine has been demonstrated to have analgesic activity and the ability to penetrate the brain of rats.[15] If, in fact, the morphine-6-glucuronide penetrated the brain and contributed to the analgesic effect, the degree of tolerance development (estimated on the basis of divergence of morphine concentration and analgesic effect) might actually have been even higher than that demonstrated in our experiments.

It should be noted that with a drug given intravenously by a constant rate of infusion, as was done in the current study, the drug concentration should be increased gradually for a period of time equal to four to five half-lives.[16] In the case of morphine, this period is more than 8 h. Thus, the absence of decline in morphine concentrations that we observed with the infusion experiments is not surprising.

Acute tolerance to the anesthetic effect of opioids has been demonstrated in animal experiments.[17,18] In patients, a study by McQuay *et al.*[19] demonstrated that the intraoperative use of fentanyl (up to 25 mg/kg) results in the development of acute tolerance to the analgesic effect of this agent in the immediate postoperative period. However, the authors of that study could not demonstrate that the patients with a higher intraoperative dose of fentanyl had required more analgesic postoperatively.

It should be mentioned that the doses used in the current study were not high enough to provide maximal analgesic effect. To prevent movement in response to the tail clamp (strong analgesia), the doses should be two to four times as high, and to produce unresponsiveness to the tail clamp and loss of the righting reflex (anesthesia), they should be five to ten times as high.[20,21] Thus, the level of analgesia in rats used in the current study should be graded as moderately high.

Patient-controlled analgesia with or without basal infusion of morphine is now commonly used for postoperative pain relief. The phenomenon of acute tolerance to the analgesic effect of morphine observed with continuous infusion in animals might well be expected in patients. However, as yet there is no published information in this regard.

In summary, with the constant-rate morphine infusion, the peak concentration of analgesia could not be maintained. It began to decline within several hours after infusion was begun, and was profoundly reduced by 8 h,

despite the absence of any decrease in the morphine brain concentration. With the single morphine injection, recovery from analgesia developed at a much faster rate than the morphine brain concentration declined. These results suggest that acute pharmacodynamic tolerance is the underlying mechanism for both phenomena.

References

1. Nordberg G, Borg L, Hedner T, Mellstrand T: CSF and plasma pharmacokinetics of intramuscular morphine. Eur J Clin Pharmacol 27:677–681, 1985
2. Jaffe JH, Martin WR: Opioid analgesics and antagonists, Goodman and Gilman's The Pharmacological Basis of Therapeutics, 7th edition. Edited by Gilman AG, Goodman LS, Rall TW, Murad F. New York, MacMillan Publishing Company, 1985, p 505
3. Mule SJ, Woods LA: Distribution of N-C^{14}-methyl labeled morphine: I. In central nervous system of nontolerant and tolerant dogs. J Pharmacol Exp Ther 136:232–241, 1962
4. Nishitateno K, Ngai SH, Finck AD, Berkowitz: Pharmacokinetics of morphine. ANESTHESIOLOGY 50:520–523, 1979
5. Hug CC, Jr., Murphy MR, Rigel EP, Olsen WA: Pharmacokinetics of morphine injected intravenously into the anesthetized dog. ANESTHESIOLOGY 54:38–47, 1981
6. Martin WR, Eades CG: Demonstration of tolerance and physical dependence in the dog following a short-term infusion of morphine. J Pharmacol Exp Ther 133:262–270, 1961
7. Cox BM, Ginsburg M, Osman OH: Acute tolerance to narcotic drugs in rats. Br J Pharmacol Chemother 33:245–256, 1968
8. Green AF, Young PA: A comparison of heat and pressure analgesiometric methods in rats. Br J Pharmacol 6:572–585, 1951
9. Edwards DJ, Popovsky Z, Bauman TJ, Bivins BA: Specific ^{125}I radioimmunoassay for morphine. Clin Chem 32:157–158, 1986
10. Snedecor GW, Cochran WG: Statistical Methods, 7th edition. Ames, The Iowa State University Press, 1980, pp 215–223, 233–237
11. Dahlström BE, Paalzow LK: Pharmacokinetics of morphine in plasma and discrete areas of the rat brain. J Pharmacokin Biopharm 3:293–302, 1975
12. Dahlström BE, Paalzow LK: Pharmacokinetics of morphine in relation to analgesia, Factors Affecting the Action of Narcotics. Edited by Adler ML, Manara L, Samanin R. New York, Raven Press, 1978, pp 233–246
13. Hipps PP, Eveland MR, Meyer ER, Sherman WR, Cicero TJ: Mass fragmentography of morphine: Relationship between brain levels and analgesic activity. J Pharmacol Exp Ther 196:642–648, 1976
14. Brown CE, Roerig SC, Burger VT, Cody RB, Fugimoto JM: Analgesic potencies of morphine 3- and 6-sulfates after intracerebroventricular administration in mice. J Pharmacol Sci 74: 821–824, 1985
15. Yoshimura H, Ida S, Oguri K, Tsukamoto H: Biochemical basis for analgesic activity of morphine-6-glucuronide. Penetration of morphine-6-glucuronide in the brain of rats. Biochem Pharmacol 22:1423–1430, 1973
16. Benet LZ, Sheiner LB: Pharmacokinetics, Goodman and Gilman's The Pharmacological Basis of Therapeutics, 7th edition. Edited by Gilman AG, Goodman LS, Rall TW, Murad F. New York, MacMillan Publishing Co, 1985, p 28
17. Askitopoulon H, Whitwam JG, Al-Khudhairi P, Chakrabarti M, Bower S, Hull CJ: Acute tolerance to fentanyl anesthesia in dogs. ANESTHESIOLOGY 63:255–261, 1981
18. Hall RI, Murphy MR, Hug CC, Jr.: The enflurane sparing effect of sufentanil in dogs. ANESTHESIOLOGY 67:518–525, 1987
19. McQuay HJ, Bullingham RES, Moor RA: Acute opiate tolerance in man. Life Sci 28:2513–2517, 1981
20. Person DL, Kissin I, Brown PT, Xavier AV, Vinik HR, Bradley EL: Morphine–caffeine analgesic interaction in rats. Anesth Analg 64:851–856, 1985
21. Kissin I, Kerr CR, Smith LR: Assessment of anaesthetic action of morphine and fentanyl in rats. Can Anaesth Soc J 30:623–628, 1983