Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 527 (2005) 71 – 76
www.elsevier.com/locate/ejphar

Development of antinociceptive tolerance and physical dependence following morphine i.c.v. infusion in mice

Natalie R. Lenard *, Sandra C. Roerig

Louisiana State University Health Sciences Center-Shreveport, Department of Pharmacology, Toxicology, and Neuroscience, United States

Received 23 December 2004; received in revised form 5 October 2005; accepted 11 October 2005

Abstract

The chronic i.c.v. infusion of morphine has been reported for rats but not for mice. In the current report, the antinociceptive tolerance to both i.c.v. morphine infusion and s.c. implantation of morphine pellets in mice was compared. Physical dependence after i.c.v. morphine infusion was also evaluated. Osmotic minipumps were filled with morphine (50 mM), connected to i.c.v. cannulae, and implanted s.c. to deliver 50 nmol/h for 3 days (i.e., 3.6 μmol total). Robust jumping precipitated by naloxone (1 mg/kg, s.c.) indicated the development of physical dependence. Tolerance to i.c.v., i.t., and i.v. morphine (6.3-, 2.0-, and 4.4-fold, respectively) was observed using the tail flick test. Mice implanted with pellets containing 75 mg morphine for 3 days (i.e., ~260 μmol total) were also tolerant to morphine (6.5-, 7.5- and 18-fold, respectively). Thus, the tolerance developed using the two methods was not identical. These results allow comparison of morphine tested by 3 different routes (i.c.v., i.t., and i.v.) after chronic morphine treatment by two routes (i.c.v. and s.c.) in a single study.

Keywords: Intracerebroventricular; Osmotic minipump; Morphine pellet; Chronic; Naloxone; Withdrawal; Tolerance; (Mice)

1. Introduction

The development of tolerance and physical dependence to morphine in both rats and mice are commonly achieved using morphine pellets implanted under the skin. There are also studies demonstrating that, in rats, both tolerance and physical dependence develop to morphine infused chronically using osmotic minipumps connected to i.c.v. cannulae (Huffman et al., 1985; Narita et al., 1994; Tokuyama et al., 2000). Mice, on the other hand, have been infused i.c.v. with neuropeptide Y (Mashiko et al., 2003), beta-amyloid (Permanne et al., 2002), melanin-concentrating hormone (Gomori et al., 2003), and other compounds, but there are no reports of morphine i.c.v. infusion in mice.

Osmotic minipumps are useful for the continuous administration of drugs and/or hormones because stable drug concentrations can be rapidly achieved and there is no stress associated with repeated injections. The use of osmotic minipumps is ideal for drugs that produce physical dependence (e.g., morphine) because continuously infused animals do not undergo periodic withdrawal during the times between injections. To eliminate the confounding factor of drug access to brain opioid receptors following systemic administration, the continuous i.c.v. infusion of morphine is a good model for the study of the development of tolerance and physical dependence.

In the current studies, we demonstrated that mice infused with morphine i.c.v. for 3 days developed antinociceptive tolerance to i.c.v., i.t., or i.v. administered morphine, as well as physical dependence. In studies comparing chronic i.c.v. infusion to chronic s.c. administration (i.e., s.c. morphine pellets), the tolerance i.c.v. morphine produced by morphine pellet implantation was similar to that produced by i.c.v. infusion of morphine. However, animals implanted with morphine pellets developed a greater degree of tolerance to i.t. and i.v. morphine than animals that had been infused with i.c.v. morphine. These data will likely be useful for other investigators because 2 different routes of chronic administration and 3 different routes of acute administration are compared using the same experimental conditions.

* Corresponding author. Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70809. Tel.: +1 2257633164; fax: +1 2257630274.
E-mail address: lenardnr@pbrc.edu (N.R. Lenard).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.10.031

2. Materials and methods

2.1. Animals

Male ICR mice (Harlan, Indianapolis, IN) that weighed 28–45 g were used throughout these studies. The animals were housed in groups of 5 at an ambient temperature of 22–23 °C in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited animal care facility under a 12–12 h light/dark cycle. Both food and water were available ad libitum, except during the testing for physical dependence. All procedures complied with the European Community guidelines for the use of experimental animals and were approved by the LSUHSC-S Animal Care and Use Committee.

2.2. Acute drug administration

Drugs were dissolved in sterile saline (0.9% NaCl). For i.c.v. injections, animals were briefly anesthetized with halothane and drugs were administered with a Hamilton syringe mated to a shortened 27-g needle in a volume of 4 μl by i.c.v. injection into the lateral cerebral ventricle (Haley and McCormick, 1957). The injection site was 1.6 mm lateral and 0.6 mm caudal to bregma, 3 mm deep. For i.t. injections, drugs were administered with a Hamilton syringe mated to a 30-g needle in a volume of 5 μl into the subarachnoid space between L5 and L6 of the spinal cord (Hylden and Wilcox, 1980). For i.v. injections, drugs were administered with a 1-ml syringe and a 27-g needle in a volume of 100 μl into a lateral tail vein.

2.3. Antinociceptive testing

Antinociception was evaluated by the radiant heat tail flick assay (D'Amour and Smith, 1941). Briefly, a beam of light was focused on the mouse tail and the time until the tail flicked was measured. Each animal served as its own control and was used only once. Mice were tested once before injection (control time). After injection, the mice were tested at the time of peak drug response (drug time), as determined by pilot time course studies (i.c.v.: 30 min; i.t.: 10 min; i.v.: 15 min). The light intensity was adjusted so that control times were between 1.5 and 2.5 s. A 10-s cutoff drug time was set to minimize the risk of tissue damage. Percent maximum possible effect was calculated as follows (Dewey et al., 1970):

$$\frac{\text{Drug time (s)} - \text{Control time (s)}}{\text{10 s} - \text{Control time (s)}} \times 100\% = \%\ \text{maximum possible effect.}$$

Graded dose response curves of at least 4 doses with 7–10 mice per dose were generated from the percent maximum possible effect data, except in the case of Fig. 1, in which 4–6 mice per dose were used in the infused animals. ED_{50} values with 95% confidence intervals were computed with GraphPad Prism using nonlinear regression methods.

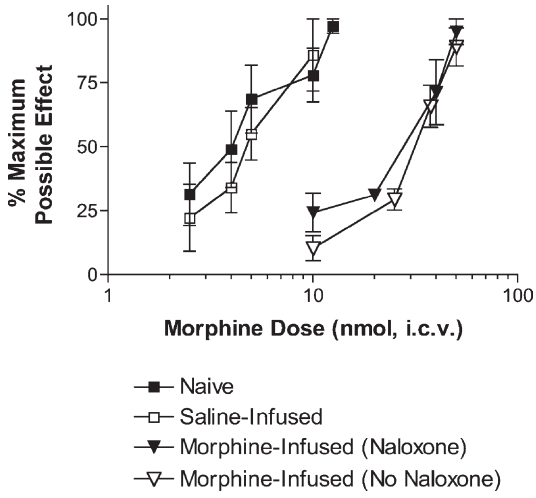

Fig. 1. Dose response for acute i.c.v. morphine in naïve mice (■) and mice infused i.c.v. with saline (□) or morphine (▼). The saline (□)- and morphine (▼)-infused mice underwent naloxone-precipitated withdrawal 3–4 h before the morphine was administered. The second group of morphine-infused mice (▽) did not undergo naloxone-precipitated withdrawal before the morphine was administered. Each point represents the mean±S.E.M of 7–10 mice per dose for the naïve mice and 4–6 per dose for the infused mice.

2.4. Preparation of the cannulae and minipumps

Osmotic minipumps (100 μl, model 1003D, Alzet, Durect Corporation, Cupertino, CA) were filled with sterile saline (0.9% NaCl) or morphine dissolved in sterile saline. The minipumps were connected by a 1.6–1.8 cm length of PE-60 tubing to 3 mm-long cannulae (osmotic pump connector cannulae, Plastics One, Roanoke, VA) and primed in sterile saline at 37 °C overnight. The dose of morphine chosen for the remaining studies (50 nmol/h) was approximately 12 times the acute injection ED_{50} value (4.13 (3.70–4.57) nmol)). In pilot experiments, 6 times the ED_{50} (25 nmol/h) infused for 3 or 7 days begat neither tolerance nor physical dependence.

2.5. Implantation of the minipumps

On the day following the minipump priming, the mice were anesthetized with Avertin (2,2,2-tribromoethanol (370 mg/kg, i.p.)/*tert* amyl alcohol (0.16 ml/kg, i.p.), Sigma, St. Louis, MO) before surgery. The scalp was shaved, Betadine® was applied, and an incision was made along the midline of the scalp; hemostats were then used to make a pocket under the skin between the shoulder blades. The skull was scraped clean of periosteum so that the cannulae would properly adhere to the skull. A micro drill (Fine Science Tools Inc., Foster City, CA) was used to create a hole approximately 1.6 mm lateral and 0.6 mm caudal to bregma. The minipump was placed in the pocket under the skin between the shoulder blades, the cannula was inserted into the drilled hole into the lateral ventricle, and the cannula pedestal was affixed to the skull with cyanoacrylate glue. The incision was closed with cyanoacrylate glue and antibacterial ointment was applied to the wound. The animals were allowed to recover on a heating pad (Fine Science Tools, Foster City, CA) and were returned to their cages in the animal

facility for 3 days. On the fourth day after surgery, and after all testing, cannula placement into the lateral ventricle was verified with trypan blue (1%, 4 μl) if the mice showed signs of neither tolerance nor dependence on the test day.

2.6. Implantation of the morphine pellets

One 150-mg pellet containing 75 mg morphine base was implanted under the dorsal skin (s.c.) of each animal (Way et al., 1969), and the incision was closed with either wound clips (9-mm autoclips, Becton–Dickinson) or cyanoacrylate glue. The mice were returned to their cages in the animal facility for 3 days. On the test day, pellets were removed 3–4 h before morphine challenge. These mice were not subject to naloxone-precipitated withdrawal.

2.7. Testing for the development of physical dependence

The development of physical dependence was assessed by counting the number of times each mouse jumped during withdrawal precipitated by naloxone (Way et al., 1969). Two groups of mice underwent this testing, i.e., the i.c.v. saline-infused mice and one group of i.c.v. morphine-infused mice (Fig. 1). On the fourth day after implantation of the osmotic minipumps, mice were injected with naloxone (1 mg/kg, s.c.) and placed into a Plexiglas® cylinder for 10 min. During that 10 min, the vertical jumps were counted as withdrawal signs. A jump was counted only when all four feet came off the floor of the cylinder. Occasionally, wet dog shakes were also observed but not quantified.

2.8. Testing for the development of antinociceptive tolerance

After the dependence testing, the saline- and morphine-infused mice were lightly anesthetized with halothane and the osmotic minipumps were removed. The mice were returned to their cages for 3–4 h. To test the degree of tolerance developed, the mice were injected with i.c.v. morphine and antinociception was measured with the tail flick test as described above (Fig. 1). In separate experiments, mice infused with i.c.v. morphine were not injected with naloxone and tolerance to i.c.v., i.t. or i.v. morphine was measured with the tail flick test (Fig. 1, Table 1).

2.9. Drugs

Morphine sulfate and morphine base pellets (75 mg morphine) were obtained from the National Institute on Drug Abuse. Naloxone hydrochloride was obtained from Sigma–Aldrich (St. Louis, MO).

2.10. Statistical analyses

ED_{50} values were considered significantly different when the 95% confidence intervals did not overlap. Withdrawal jumping of morphine-infused animals was compared with to that of saline-infused animals with a two-tailed Students' *t*-test with Welch's correction for unequal variances. Significance was accepted at $P<0.05$.

Table 1
Effect of saline or morphine i.c.v. infusion and morphine s.c. pellets on the potency of acute i.c.v., i.v., and i.t. morphine

	Morphine ED_{50} (95% CI)		
	i.c.v. (nmol)	i.t. (pmol)	i.v. (nmol)
Naïve mice	4.13 (3.70–4.57)	151 (142–158)	168 (146–178)
Saline i.c.v. infusion	5.12 (3.65–6.60)	171 (147–179)	195 (85–375)
Morphine i.c.v. infusion	32.0 (28.7–35.3)	349 (319–357)	861 (488–1483)
Morphine s.c. pellets	26.7 (22.4–31.0)	1132 (444–2203)	3093 (2836–3302)

These mice did not receive naloxone treatment. The ED_{50} values for acute i.c.v. morphine in naïve and saline-infused animals are from Fig. 1. Each ED_{50} value was determined from a dose response curve of at least four doses with at least six mice per dose.

3. Results

3.1. Effect of acute i.c.v. morphine in naïve animals

Morphine was injected into the right lateral cerebral ventricle and the tail flick responses were measured 30 min later. A dose response curve was generated and the ED_{50} value was determined using nonlinear regression methods. The ED_{50} value for i.c.v. morphine in naïve animals was 4.13 (3.70–4.57) nmol (Fig. 1, Table 1).

3.2. Overall effects of the chronic i.c.v. infusion surgery

On the afternoon following the i.c.v. cannulation surgery performed in the morning, the morphine-infused mice exhibited the Straub tail reaction. Otherwise, behavior appeared normal and not different from mice infused i.c.v. with saline. The morphine-infused mice lost an average of 3 g (range: 0–10 g) body weight during the 3-day infusion period. Presumably, the high dose of morphine infused inhibited the animals' appetites. This is in contrast to saline-infused mice, whose weights did not change markedly (average change +1 g; range: −1 +2 g). Also, morphine-infused mice did not groom themselves like the saline-infused mice did, resulting in scruffy fur on the fourth day after the surgery. No motor impairments were observed at any time.

3.3. Effect of naloxone in mice infused i.c.v. with saline or morphine for 3 days

On the fourth day after surgery, mice were injected with naloxone (1 mg/kg, s.c.) and immediately placed into a Plexiglas® cylinder. The morphine-infused mice jumped an average of 100 (±15) times in the 10-min observation period. Unexpectedly, the saline-infused mice did exhibit some jumping behavior, although it was much less robust than that of the morphine-infused mice (6.3±5.0 times/10 min). Some morphine-infused animals displayed wet dog shakes, although only

as many as 5 times during the 10-min observation period. Other withdrawal signs reported for rats, such as teeth chattering, piloerection, and ptosis (Wei, 1973), were not observed in the mice. Importantly, no evidence of withdrawal-induced diarrhea was observed after the 3-day i.c.v. morphine treatment, as has been reported for rats (Ho et al., 1979).

3.4. Effect of acute i.c.v. morphine in mice infused i.c.v. with saline or morphine for 3 days

After the dependence testing was completed, the minipumps were removed and the animals were returned to their cages for 3–4 h. Morphine was injected into the contralateral lateral cerebral ventricle (i.e., the ventricle in which the cannula was not located) and tail flick responses were measured 30 min later. The ED_{50} value for i.c.v. morphine in saline-infused animals was not different from that of naïve animals (i.e., 5.12 (3.65–6.60) nmol vs. 4.13 (3.70–4.57) nmol) (Fig. 1, Table 1). In morphine-infused animals that also received naloxone, however, the dose response curve was shifted to the right. The ED_{50} value for i.c.v. morphine (26.8 (20.8–32.8) nmol, Fig. 1) was approximately 5-fold greater than that of saline-infused animals (5.12 (3.65–6.60) nmol, Fig. 1, Table 1). It should be noted that these mice had been injected with naloxone. In separate experiments, in which mice infused i.c.v. with morphine did not receive naloxone, similar ED_{50} values were obtained. The ED_{50} value for i.c.v. morphine in these mice was 32.0 (28.7–35.3) (Fig. 1, Table 1). This ED_{50} value was not different from the value obtained using mice infused with morphine i.c.v. and subject to naloxone-precipitated withdrawal (26.8 (20.8–32.8) nmol (Fig. 1).

3.5. Effect of acute i.t. or i.v. morphine in naïve mice compared to mice infused i.c.v. with saline or morphine for 3 days

Another group of mice was infused i.c.v. with either saline or morphine for 3 days . On the fourth day, the minipumps were removed and the mice were returned to their cages for 4 h. Morphine was injected i.t. or i.v. and tail flick responses were measured 10 or 15 min later, respectively. The ED_{50} value for i.t. morphine in saline-infused animals was not different from that of naïve animals (Table 1). The morphine dose response curve for morphine-infused mice, however, was shifted to the right. The ED_{50} value for i.t. morphine was approximately twice than that of saline-infused animals (Tables 1 and 2). For the i.v. tolerance studies, the ED_{50} value for i.v. morphine in saline-infused animals was not different from that of naïve animals (Table 1). The ED_{50} value for i.v. morphine in morphine-infused animals, however was approximately 4 times that of saline-infused animals (Tables 1 and 2).

Table 2
The fold tolerance resulting from either the i.c.v infusion of morphine or the s.c. implantation of morphine pellets

	Fold tolerance		
	i.c.v.	i.t.	i.v.
Morphine i.c.v. infusion	6.3	2.0	4.4
Morphine s.c. pellets	6.5	7.5	18.0

The fold tolerance is calculated by dividing the ED_{50} value obtained after morphine treatment by the ED_{50} value obtained using i.c.v. saline-infused mice in the i.c.v. infusion experiments and by the ED_{50} value obtained using naïve mice in the s.c. pellet experiments.

3.6. Effect of acute i.c.v., i.t., or i.v. morphine in mice implanted s.c. with morphine pellets for 3 days

In another group of mice, morphine pellets were implanted under the dorsal skin. On the fourth day following implantation, the pellets were removed and the mice were returned to their cages for 3–4 h (Lange et al., 1980). Morphine was injected i.c.v., i.t., or i.v., and tail flick responses were measured at the time of peak drug response. The ED_{50} values for i.c.v., i.t., and i.v. morphine were all different from the values obtained using naïve mice (Table 1). Strikingly, the ED_{50} values for i.c.v. morphine were similar whether the mice were chronically infused i.c.v. with morphine or implanted s.c. with morphine pellets (Tables 1 and 2). Whereas the ED_{50} value for i.t. morphine in the i.c.v. infused mice was approximately twice that of naïve or saline-infused animals, the i.t. ED_{50} value obtained from pellet-implanted animals was 7.5 times higher than that of naïve animals (Tables 1 and 2). The s.c. morphine pellets also produced a rightward shift in the dose response curve for systemically administered (i.v.) morphine, as expected. The ED_{50} value for i.v. morphine in pellet implanted mice was approximately 18 times that of naïve mice (Tables 1 and 2). Interestingly, in naïve animals, i.v. morphine (ED_{50} ~1.8 (1.6–1.9) mg/kg) was more potent when compared with s.c. morphine (ED_{50}=4.5 (3.3–6.2) mg/kg) reported previously by Lange et al. (1980). However, this difference in potency was no longer apparent in morphine pellet implanted mice. The i.v. morphine ED_{50} value was approximately 33 (31–36) mg/kg, similar to the s.c. morphine ED_{50} value (32.5 (20.6–51.3) mg/kg) reported by Lange et al. (1980).

4. Discussion

These studies describe procedures for the 3-day continuous infusion of morphine into the right lateral cerebral ventricles of mice. These mice developed both physical dependence and tolerance to morphine administered by 3 different routes. The tolerance results are compared to data obtained with mice implanted s.c. with morphine pellets for 3 days.

Mice infused i.c.v. with high doses (i.e., ~12× ED_{50}) of morphine developed tolerance and physical dependence. Interestingly, the morphine i.c.v. infusion produced the same degree of tolerance to i.c.v. morphine as did the s.c. morphine pellets. Also, i.c.v. infusion of morphine produced a 2.0-fold tolerance to i.t. morphine, suggesting that the morphine infused i.c.v. diffused caudally to the spinal cord to render spinal opioid sites tolerant. The degree of i.t. tolerance resulting from the morphine i.c.v. infusion is substantially lower than that produced by the s.c. morphine pellets (2.0-fold vs. 7.5-fold, respectively). One possible explanation for this difference is that more morphine reaches the spinal sites with morphine pellets

than it does with i.c.v. infusion of morphine and its subsequent diffusion to the spinal cord. Exposure to a higher concentration of the drug would likely produce a greater degree of tolerance. Evidence for this association can be found in our own studies because pilot experiments in which chronic i.c.v. infusion of 6× the morphine ED_{50} value did not produce tolerance to i.c.v. morphine, whereas 12× the ED_{50} value produced 6.3-fold tolerance.

Previous studies utilizing intermittent dosing instead of osmotic minipumps have also evaluated the development of antinociceptive tolerance to i.c.v. administered morphine in mice. In one example, morphine administered i.c.v. on an escalating daily dose schedule 3 times daily for 3 days resulted in the development of 7.8-fold tolerance in male CD-1 mice (Kest and Hopkins, 2001). These data are similar to our results (i.e., 7.8-fold vs. 6.3-fold), even though less total morphine was administered (i.e., 0.7 vs. 3.6 μmol). It is likely that the escalating dosing schedule accounts for this difference. Also, the phenomenon of withdrawal tolerance may be involved in a protocol utilizing intermittent morphine injections (Lange et al., 1983). In other studies, male Swiss Webster mice were administered morphine at a dose approximately 4× ED_{50} (30 nmol) once daily for 5 days (Soignier et al., 2004). Tolerance to morphine developed by the third day even though much less morphine was administered (i.e. 0.09 vs. 3.6 μmol). Because this group did not construct dose response curves, it is impossible to determine whether the fold tolerance induced by their protocol is similar to our results. We and others have shown strain differences in the development of tolerance to morphine that have been documented (Roerig and Fujimoto, 1988; Kest et al., 2002a), which may account for differences in amount of morphine required to induce tolerance.

The 3-day i.c.v. infusion of morphine in mice can be compared to similar experiments performed in rats. Physical dependence developed following the morphine i.c.v. infusion in mice, as has been observed in rats (Huffman et al., 1985; Feng et al., 1994). Withdrawal precipitated by naloxone in dependent rats is characterized by the following signs: exploring, teeth chattering, whole-body or wet dog shakes, and jumping (Wei, 1973) and is sometimes represented as a composite score. Because the strongest withdrawal sign seen in mice is jumping, it is difficult to compare directly the degree of dependence developed between rats and mice. It is important to point out, however, that the predominant withdrawal sign in rats with a very high degree of dependence is jumping (Bhargava, 1977; Roerig et al., 1985).

In experiments in rats, Vonhof et al. (2003) infused morphine (30 nmol/h) i.c.v. for 6 days using osmotic minipumps. Antinociceptive tolerance developed and naloxone (20 mg/kg, i.p.) elicited significantly more withdrawal signs in these animals than did saline. Other investigators (Feng et al., 1996) using a similar protocol (26 nmol/h morphine for 3 days) showed that the mu opioid receptor-selective antagonist D-Phe–Cys–Tyr–D-Trp–Orn–Thr–Pen–Thr–NH_2 (CTOP) or the delta selective antagonist naltrindole elicited more withdrawal signs than did saline. The same investigators reported (Feng et al., 1994) that, using the same protocol, the rats developed 46-fold antinociceptive tolerance to morphine. Clearly, the tolerance developed in these rats was greater than that developed in the mouse model presented here (i.e., 6.3-fold tolerance). Tolerance to i.c.v. morphine is likely to develop differently between the two species, accounting for the contrasting fold tolerances observed in mice vs. reported in rats.

The physical dependence developed during i.c.v. infusion of morphine in mice observed in the current studies can be compared to the development of physical dependence following implantation of morphine pellets in mice. Using morphine pellets, Vela et al. (1995) showed that naloxone (1 mg/kg, i.p.) induced approximately 50 jumps in a 30-min period, somewhat fewer than were observed with naloxone (1 mg/kg) injected s.c. (i.e., 100 jumps in a 10-min period) in mice infused i.c.v. with morphine (current studies). The route of administration for naloxone may account for this difference. Also, strain differences in the development of physical dependence to morphine have been reported (Kest et al., 2002b), perhaps explaining differences between our studies and the results from Vela et al. (1995). Similar to the current report, however, in mice made acutely tolerant to morphine with a single injection (50 mg/kg, s.c.), naloxone precipitated approximately 105 jumps in a 15-min period (Kest et al., 1996) and naltrexone (50 μg/kg, s.c.) induced approximately 100 jumps in 15 min, (Thorat et al., 1994).

The degree of tolerance developed to morphine infused i.c.v. in mice can also be compared to existing literature examining the development of tolerance using morphine pellets in mice. Lange et al. (1980) demonstrated that 3-fold tolerance developed to morphine administered i.c.v. 3 h following removal of the morphine pellets. Similarly, the present studies show that mice either infused i.c.v. with morphine or implanted with morphine pellets were 6.3–6.5-fold tolerant to i.c.v. morphine after minipump or pellet removal (3–4 h). In comparison, others have reported 14-fold tolerance to i.c.v. administered morphine in mice 4 h after pellet removal (Tseng et al., 1993).

The i.v. tolerance developed to the s.c. morphine pellets appeared to be greater than expected. In the study by Lange et al. (1980) mentioned in the previous paragraph, 7-fold tolerance developed to systemically administered (s.c.) morphine 3 h after morphine pellet removal. In contrast, we found 18-fold tolerance to systemically administered (i.v.) morphine in similarly treated animals. The mechanism underlying the differential tolerance to s.c. vs. i.v. administered morphine remains to be elucidated, but likely involves the route of acute administration.

The methods described for i.c.v. infusion are likely to be practical for studies that require infusion for longer than 3 days. We have used larger minipumps (200 μl, Alzet model 2001) for 7-day continuous infusions of morphine. However, at the lower dose of morphine used (i.e., 25 vs. 50 nmol/h), the mice did not develop tolerance or physical dependence with the 7-day i.c.v. infusion.

In summary, we present methods for the chronic i.c.v. infusion of morphine in mice. The mice developed both physical dependence and antinociceptive tolerance, and the tolerance data were compared to the tolerance developed when mice are implanted with morphine pellets. First, because a fairly small

volume is needed to fill minipumps, the i.c.v. infusion techniques are convenient for the chronic administration of either expensive drugs or drugs of limited availability. These procedures can also likely be applied to other opioids besides morphine or other classes of drugs that produce tolerance and/or physical dependence. Second, these findings are important because the tolerance developed to acute morphine administered by 3 different routes following morphine administered chronically by 2 different routes is compared in a single study.

Acknowledgements

We thank Emily Collins and Justin Moore for expert technical assistance, and Dr. Carolyn Fairbanks (University of Minnesota) for helpful suggestions. These studies were supported by NIH grants DA15091 and DA18028.

References

Bhargava, H.N., 1977. Rapid induction and quantitation of morphine dependence in the rat by pellet implantation. Psychopharmacology (Berl) 52, 55–62.

D'Amour, F.E., Smith, D.L., 1941. A method for determining loss of pain sensation. J. Pharmacol. Exp. Ther. 72, 74–79.

Dewey, W.L., Harris, L.S., Howes, J.F., Nuite, J.A., 1970. The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. J. Pharmacol. Exp. Ther. 175, 435–442.

Feng, Y.Z., Tseng, Y.T., Jaw, S.P., Hoskins, B., Ho, I.K., 1994. Tolerance development to butorphanol: comparison with morphine. Pharmacol. Biochem. Behav. 49, 649–655.

Feng, Y.Z., Zhang, T., Tokuyama, S., Zhu, H., Rockhold, R.W., Ho, I.K., 1996. Mu- and delta-opioid receptor antagonist precipitate similar withdrawal phenomena in butorphanol and morphine dependence. Neurochem. Res. 21, 63–71.

Gomori, A., Ishihara, A., Ito, M., Mashiko, S., Matsushita, H., Yumoto, M., Tanaka, T., Tokita, S., Moriya, M., Iwaasa, H., Kanatani, A., 2003. Chronic intracerebroventricular infusion of MCH causes obesity in mice. Melanin-concentrating hormone. Am. J. Physiol. Endocrinol. Metab. 284, E583–E588.

Haley, T.J., McCormick, W.G., 1957. Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse. Br. J. Pharmacol. 12, 12–15.

Ho, A.K., Chen, R.C., Kreek, M.J., 1979. Morphine withdrawal in the rat: assessment by quantitation of diarrhea and modification by ethanol. Pharmacology 18, 9–17.

Huffman, R.D., Simmons, K.E., Lum, J.T., 1985. An intraventricular infusion model for inducing morphine dependence in rats: quantitative assessment of precipitated withdrawal. Behav. Neurosci. 99, 861–880.

Hylden, J.L., Wilcox, G.L., 1980. Intrathecal morphine in mice: a new technique. Eur. J. Pharmacol. 67, 313–316.

Kest, B., Hopkins, E., 2001. Morphine tolerance after chronic intracerebroventricular injection in male and female mice. Brain Res. 892, 208–210.

Kest, B., Lee, C.E., McLemore, G.L., Inturrisi, C.E., 1996. An antisense oligodeoxynucleotide to the delta opioid receptor (DOR-1) inhibits morphine tolerance and acute dependence in mice. Brain Res. Bull. 39, 185–188.

Kest, B., Hopkins, E., Palmese, C.A., Adler, M., Mogil, J.S., 2002a. Genetic variation in morphine analgesic tolerance: a survey of 11 inbred mouse strains. Pharmacol. Biochem. Behav. 73, 821–828.

Kest, B., Palmese, C.A., Hopkins, E., Adler, M., Juni, A., Mogil, J.S., 2002b. Naloxone-precipitated withdrawal jumping in 11 inbred mouse strains: evidence for common genetic mechanisms in acute and chronic morphine physical dependence. Neuroscience 115, 463–469.

Lange, D.G., Roerig, S.C., Fujimoto, J.M., Wang, R.I.H., 1980. Absence of cross-tolerance to heroin in morphine-tolerant mice. Science 208, 72–74.

Lange, D.G., Roerig, S.C., Fujimoto, J.M., Busse, L.W., 1983. Withdrawal tolerance and unidirectional non-cross-tolerance in narcotic pellet-implanted mice. J. Pharmacol. Exp. Ther. 224, 13–20.

Mashiko, S., Ishihara, A., Iwaasa, H., Sano, H., Oda, Z., Ito, J., Yumoto, M., Okawa, M., Suzuki, J., Fukuroda, T., Jitsuoka, M., Morin, N.R., MacNeil, D. J., Van der Ploeg, L.H., Ihara, M., Fukami, T., Kanatani, A., 2003. Characterization of neuropeptide Y (NPY) Y5 receptor-mediated obesity in mice: chronic intracerebroventricular infusion of D-Trp(34)NPY. Endocrinology 144, 1793–1801.

Narita, M., Feng, Y., Makimura, M., Hoskins, B., Ho, I.K., 1994. A protein kinase inhibitor, H-7, inhibits the development of tolerance to opioid antinociception. Eur. J. Pharmacol. 271, 543–545.

Roerig, S.C., Fujimoto, J.M., 1988. Morphine antinociception in different strains of mice: relationship of supraspinal–spinal multiplicative interaction to tolerance. J. Pharmacol. Exp. Ther. 247, 603–608.

Roerig, S.C., Fujimoto, J.M., Franklin, R.B., Lange, D.G., 1985. Unidirectional non-cross-tolerance (UNCT) in rats and an apparent dissociation between narcotic tolerance and physical dependence. Brain Res. 327, 91–96.

Permanne, B., Adessi, C., Saborio, G.P., Fraga, S., Frossard, M.J., Van Dorpe, J., Dewachter, I., Banks, W.A., Van Leuven, F., Soto, C., 2002. Reduction of amyloid load and cerebral damage in a transgenic mouse model of Alzheimer's disease by treatment with a beta-sheet breaker peptide. FASEB J. 16, 860–862.

Soignier, R.D., Vaccarino, A.L., Fanti, K.A., Wilson, A.M., Zadina, J.E., 2004. Analgesic tolerance and cross-tolerance to i.c.v. endomorphin-1, endomorphin-2, and morphine in mice. Neurosci. Lett. 366, 211–214.

Thorat, S.N., Barjavel, M.J., Matwyshyn, G.A., Bhargava, H.N., 1994. Comparative effects of NG-monomethyl-L-arginine and MK-801 on the abstinence syndrome in morphine-dependent mice. Brain Res. 642, 153–159.

Tokuyama, S., Ho, I.K., Yamamoto, T., 2000. A protein kinase inhibitor, H-7, blocks naloxone-precipitated changes in dopamine and its metabolites in the brains of opioid-dependent rats. Brain Res. Bull. 52, 363–369.

Tseng, L.F., Lin, J.J., Collins, K.A., 1993. Partial antinociceptive cross-tolerance to intracerebroventricular beta-endorphin in mice tolerant to systemic morphine. Eur. J. Pharmacol. 241, 63–70.

Vela, G., Ruiz-Gayo, M., Fuentes, J.A., 1995. Anandamide decreases naloxone-precipitated withdrawal signs in mice chronically treated with morphine. Neuropharmacology 34, 665–668.

Vonhof, S., Gudka, B.D., Siren, A.-L., 2003. Tolerance and dependence following chronic intracerebroventricular infusion of Tyr–D-Arg2–Phe–Sar4 (TAPS). Eur. J. Pharmacol. 459, 41–48.

Way, E.L., Loh, H.H., Shen, F.H., 1969. Simultaneous quantitative assessment of morphine tolerance and physical dependence. J. Pharmacol. Exp. Ther. 167, 1–8.

Wei, E., 1973. Assessment of precipitated abstinence in morphine-dependent rats. Psychopharmacologia 28, 35–44.