Pharmacology Biochemistry & Behavior, Vol. 26, pp. 131–139, 1987. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Determination of Cross Tolerance in Rat Spinal Cord Using Intrathecal Infusion Via Sequential Mini-Osmotic Pumps[1]

C. W. LOOMIS, B. MILNE AND F. W. CERVENKO

Department of Anaesthesiology and Department of Pharmacology and Toxicology
Faculty of Medicine, Queen's University, Kingston, Ontario, Canada K7L 3N6

Received 16 June 1986

LOOMIS, C. W., B. MILNE AND F. W. CERVENKO. *Determination of cross tolerance in rat spinal cord using intrathecal infusion via sequential mini-osmotic pumps.* PHARMACOL BIOCHEM BEHAV **26**(1) 131–139, 1987.— Continuous intrathecal (IT) infusion via ALZET® mini-osmotic pumps was used to induce spinal tolerance to morphine in the rat. Naloxone (1 mg/kg IP), injected on day 3 of continuous IT morphine (10 μg/hr), produced mild withdrawal symptoms in all morphine-treated animals. In rats pretreated with continuous IT morphine (10 μg/hr) or saline, systemic morphine (2, 4, 8, 10 and 15 mg/kg IP) produced equivalent, dose-dependent antinociception using the tail-flick and paw pressure tests. The rostral and caudal distribution of methylene blue dye in rat spinal cord was determined on days 1–7 of continuous IT infusion. The dye remained localized near the catheter tip throughout infusion; maximum distribution was 1.5 cm rostrally and 1.0 cm caudally. The data indicate that morphine, infused at the rate of 10 μg/hr, does not undergo extensive redistribution in the spinal cord. A sequential, double mini-osmotic pump technique for cross tolerance studies in rat spinal cord is described. In rats pretreated with continuous IT norepinephrine for 4 days, the antinociceptive actions of continuous IT morphine were reduced but not significantly different from saline-pretreated animals. These data suggest that morphine, injected into the spinal cord, does not produce behavioural analgesia by activation of local adrenergic systems.

Cross tolerance Morphine Naloxone Spinal cord Mini-osmotic pumps Antinociception

BASIC and clinical studies have shown that opioids, applied to the spinal cord, produce profound, stereospecific, naloxone-reversible analgesia [2, 29, 32, 33, 37]. These observations, and the identification of opioid receptors in the dorsal horn of the spinal cord led to the realization that opioid analgesia is mediated, in part, at the spinal level. A major advantage of spinal analgesia appears to be the control of pain without significantly disturbing motor and autonomic function [5,32]. However, the chronic administration of opioids to the spinal cord of animals and humans leads to the development of tolerance and cross tolerance to their analgesic effects [11, 31, 39]. The development of tolerance and other adverse effects (delayed respiratory depression, urinary retention, pruritis) during spinal administration currently limit the clinical usefulness of this technique.

Descending noradrenergic and serotonergic bulbospinal pathways have been identified in animals which modulate the spinal processing of nociceptive information [1,34]. Furthermore, spinal injections of the monoamines norepinephrine and serotonin evoke behavioural analgesia in animals which is attributed to the activation of α-adrenoceptors and serotonergic receptors in the dorsal horn of the spinal cord, respectively [22, 23, 35]. The ability of these agonists to produce analgesic effects via distinct receptors theoretically makes such agents useful for maintaining analgesia during opioid tolerance or for optimizing spinal analgesia. We have been investigating this hypothesis in rats chronically implanted with intrathecal (IT) catheters.

One of the main objectives of our research was to determine the extent of cross tolerance between opioid and non-opioid analgesics in the spinal cord. In animal studies of opioid tolerance, several techniques have been used to experimentally induce tolerance. These include repeated systemic or central injections according to a specific dose schedule [4,9], the implantation of a subcutaneous (SC) opioid pellet [30], the use of a slow-release SC suspension [5,10], and SC implanted mini-osmotic pumps [25]. These techniques induce tolerance to opioids at both supraspinal and spinal sites. To induce tolerance in the spinal cord, repeated IT injections of opioids have been used [39] and more

[1]Supported by the Medical Research Council of Canada.

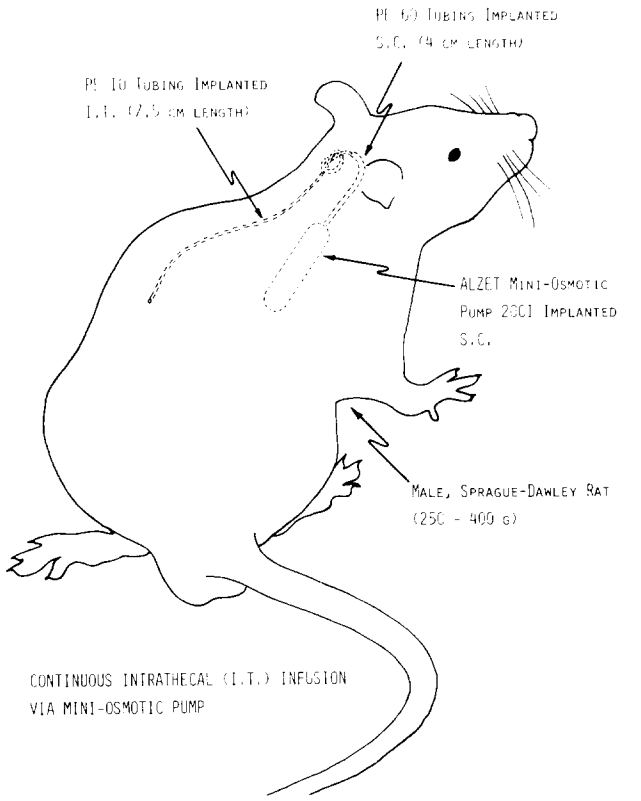

FIG. 1. A diagram illustrating the chronic IT catheter, the mini-osmotic pump, and the approximate positions of these implants on the back of the rat. The abbreviations SC and IT represent subcutaneous and intrathecal, respectively.

recently, continuous microperfusion into the spinal cord of the rat using mini-osmotic pumps has been reported [26,31]. In this paper, we describe a technique using two ALZET® mini-osmotic pumps in sequence for the determination of spinal cross tolerance in the rat. The characteristics of analgesia and tolerance during continuous IT infusion using single osmotic minipumps were investigated first to determine the reliability of this technique for delivering drug to the spinal cord. The sequential double pump technique was then used in cross tolerance studies of IT morphine and norepinephrine in the rat.

METHOD

Construction of the Intrathecal (IT) Catheter

The IT catheter was constructed from two different sizes of polyethylene (PE) tubing. Using a 12-cm length of PE 10 tubing, a small loop (approximately 4 mm diameter) was made 4 cm from the end of the catheter and fixed with adhesive to prevent the loop from tightening. The short end of the PE 10 catheter was then connected to a 4-cm length of PE 60 tubing by inserting the PE 10 tubing inside the PE 60 tubing for a distance of 1.0 cm. The connection was then sealed with a commercially available adhesive (Seal All, Allen Stevenson Products, Windsor, Canada) to provide a secure but flexible joint. The PE 60 tubing is necessary to connect the flow moderator of the mini-osmotic pump to the PE 10 catheter. The long end of the PE 10 tubing was then cut to a length of 7.5 cm measured from the loop and the entire catheter was filled with sterile saline using a 50-μl syringe prior to surgical implantation. The complete IT catheter, the osmotic pump, and their approximate position in the rat are shown in Fig. 1. The dead volume of the entire catheter (PE 10 + PE 60) is approximately 23 μl.

Catheter and Pump Implantation

Male, Sprague-Dawley rats (250–350 g) were used in all experiments. Animals were allowed to acclimatize to their surroundings for at least one week prior to use. All surgical procedures were conducted under halothane anesthesia using a Fluotec halothane vaporizer. Following induction, the rat was placed in a stereotaxic apparatus for the surgical implantation of an IT catheter and anesthesia was maintained with 2.0 to 2.5 vol.% halothane in oxygen (100%). The PE 10 catheter was inserted through a small slit in the atlanto-occipital membrane according to the method of Yaksh and Rudy [38]. The catheter was passed distally in the subarachnoid space, terminating in the lumbar enlargement with the loop of the catheter resting against the atlanto-occipital membrane. Ten microliters of sterile saline were injected into the catheter to clear the catheter of blood and debris that may have accumulated during insertion. The externalized end of the catheter (PE 60) was heat-sealed and implanted in the subcutaneous space on the back of the animal. The incision was closed with silk sutures, halothane administration was discontinued and a 1-week recovery period was allowed prior to experimentation. Tap water and Purina® rodent laboratory chow 5001 were provided ad lib, the room temperature was maintained at 22°C and a 12-hr light:12-hr dark cycle was used with lights on at 0700 hr. Only those animals which were completely free of neurological sequellae, as assessed by the presence of normal motor function, normal baseline responses to nociceptive stimuli and the absence of atypical behaviour were used.

Single Pump Studies

To determine the extent of drug migration from the tip of IT catheter at various times during continuous IT infusion, ALZET® 2001 mini-osmotic pumps (flow rate 1 μl/hr; Alza Corp., Palo Alto) were filled with a sterile, 1% aqueous solution of methylthionine chloride (Methylene Blue Inj. USP; Allen & Hanburys, Toronto, Canada). On day 0, the pumps were connected to the IT catheter and implanted in the SC space on the back of the rat under halothane anesthesia as follows. The position of the catheter was located under the skin on the back of the animal by rolling the skin with the thumb and forefinger. A 2-cm incision was made through the skin adjacent to the catheter taking care not to cut or puncture the catheter. The tip of the catheter, which had been heat sealed before implantation, was externalized, cut with a pair of scissors, and connected to a minipump by inserting the tip of the pump flow moderator tube into the PE 60 tubing. The PE 60 tubing should sit tightly against the flange of the flow moderator to ensure that the connection is secure and leak-proof. The SC pocket on the back of the animal was enlarged with a pair of hemostats and the catheter and pump were inserted. The incision was closed with silk sutures, halothane was discontinued and the animal was allowed to recover. Rats were given a lethal dose of urethane by IP injection on days 1, 2, 4, 5 and 7 of continuous IT infusion and a dorsal laminectomy was performed. The spinal cord was exposed, the tip of the IT catheter was located,

TABLE 1

ROSTRAL AND CAUDAL MIGRATION OF METHYLENE BLUE IN THE SPINAL CORD OF THE RAT DURING CONTINUOUS INTRATHECAL INFUSION USING MINI-OSMOTIC PUMPS*

Duration of Continuous Infusion (Days)	Distance From Tip of Lumbar Intrathecal Catheter (cm)	
	Rostral	Caudal
1	0.25	0.25
2	1.0	1.0
4	1.25	1.0
5	1.5	1.0
7	1.5	0.5

*ALZET® 2001 mini-osmotic pumps were filled with a 1% aqueous, sterile solution of methylene blue and infused intrathecally at the rate of 1 μl/hr.

and the extent of rostral and caudal dye migration was measured from the tip of the IT catheter.

To determine if animals made tolerant to spinal morphine using continuous IT infusion were tolerant to the antinociceptive effects of systemic morphine, rats were fitted with chronic IT catheters as described above. Control analgesia testing using the tail-flick test [6] was initiated 2 days prior to implantation of the mini-osmotic pumps. Tail-flick latency was determined as previously described using a cut-off time of 10 sec [15]. On day 0, rats were randomly assigned to two treatment groups and each rat was implanted with an ALZET® 2001 mini-osmotic pump to deliver either morphine or sterile saline for 7 days. Morphine sulfate (BDH Pharmaceuticals, Toronto, Canada) was dissolved in sterile saline (10 μg/μl) and infused at the rate of 10 μg/hr. This dose was selected based on both the time couse of tail-flick latency and the maximum effect produced during continuous IT infusion. In a previous study, 10 μg/hr morphine sulfate produced a significant and sustained increase in tail-flick latency which remained below the 10 sec cutoff [17]. This hourly dose is below the ED50 for morphine sulfate in the tail-flick test following acute IT administration (14.5 μg) [15]. Analgesia testing resumed the next day and was conducted once daily to assess the effect of morphine and the onset of tolerance. Analgesia testing was conducted at the same time each morning to minimize the effects of diurnal variation. Immediately after testing on day 4, all animals received a single IP injection of morphine sulfate (10 mg/kg) and the tail-flick latency was determined at 15, 30, 45, 60, 90, 120, 150 and 180 min after injection. On day 5, all animals received an IP injection of morphine sulfate (15 mg/kg) after testing and the time course of tail-flick latency was determined. To construct dose-response curves for the antinociceptive effect of systemic morphine in IT saline- and morphine-treated rats, the experiment was repeated with a different group of animals using the tail-flick and paw pressure withdrawal test. In this experiment, 2, 4 and 8 mg/kg doses of morphine sulfate were given to all rats by IP injection on days 5, 6 and 7 of continuous IT infusion, respectively. The paw pressure withdrawal test was conducted as previously described [21]. Mechanical pressure was applied to the upper surface of the non-inflammed hind paw and was released when a withdrawal response was observed. Pressure was measured in mmHg and the maximum pressure applied was 300 mmHg. Animals could be repeatedly subjected to this test without inducing hemmorhage or inflammation of the hind paw and highly reproducible threshold values were observed. Similar results using this paw pressure technique have been reported [19].

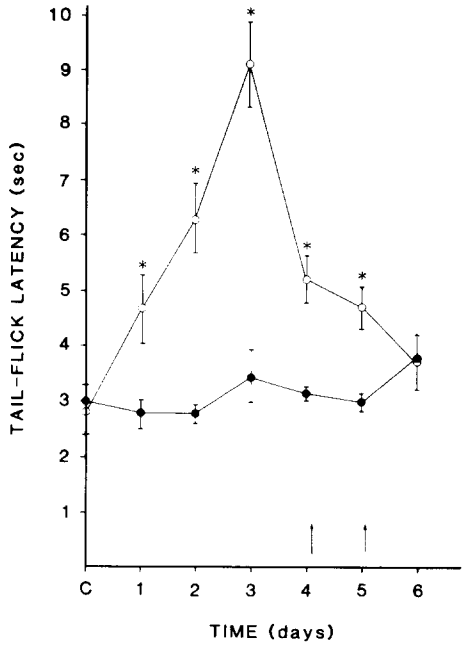

FIG. 2. Time course of the effect of continuous intrathecal infusion of morphine (○ 10 μg/hr) and saline (● 1 μl/hr) on tail-flick latency. Data are expressed as the mean±S.E.M. of 4 rats and the * denotes significant difference from control. Arrows indicate the intraperitoneal administration of 10 and 15 mg/kg morphine to both groups immediately after analgesia testing on days 4 and 5, respectively.

Double Pump Cross Tolerance Study

To determine if there is cross tolerance to morphine in rats tolerant to spinal norepinephrine (NE), animals were implanted with chronic IT catheters as described. Animals underwent control analgesia testing using the tail-flick test for 2 days. On day 0, the animals were randomly assigned to two treatment groups and implanted with ALZET® 2001 minipumps containing either NE (15 μg/μl) or vehicle. (−)Norepinephrine hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in ascorbic acid-saline (0.1 mg of ascorbic acid/ml of 0.9% saline) and infused at the rate of 15 μg/hr. This dose was selected based on the time-course of tail-flick latency and the maximum effect produced during continuous IT infusion [17]. Analgesia testing resumed the next day and was repeated once daily during infusion. Continuous IT infusion of NE was maintained until tolerance developed to the analgesic effect, as determined by a progressive decrease in tail-flick latency. Immediately after analgesia testing on day 4, the pumps containing NE or vehicle were removed under halothane anesthesia and replaced with pumps containing morphine sulfate (10 μg/μl) as described above. Analgesia testing resumed the following day and was repeated once daily for one week. Analgesia testing was conducted at the same time each day. To ensure that

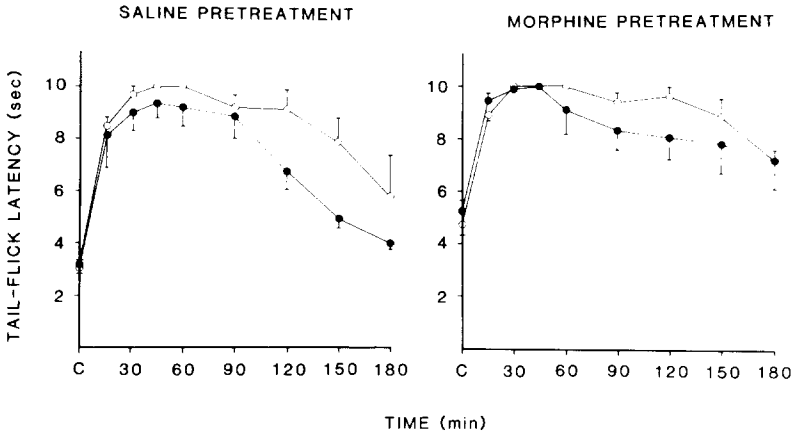

FIG. 3. Time course of the effect of intraperitoneal morphine on tail-flick latency in rats receiving continuous intrathecal infusions of saline (1 μl/hr) or morphine (10 μg/hr). Morphine (● 10 mg/kg) was injected on day 4 of continuous intrathecal infusion. Morphine (○ 15 mg/kg) was injected on day 5 of infusion. Data are expressed as the mean±S.E.M. of 4 animals. All points are significantly different from their respective controls except for 180 min in the saline group receiving 10 mg/kg morphine.

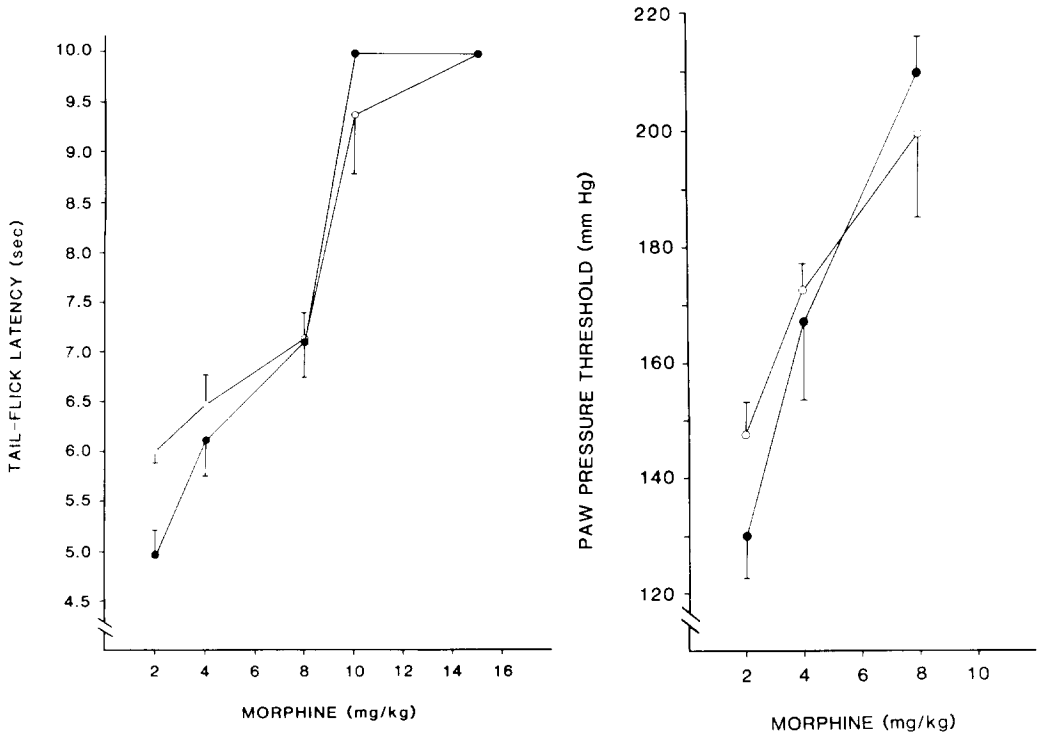

FIG. 4. The effect of intraperitoneal morphine on tail-flick latency and paw pressure threshold in rats receiving continuous intrathecal infusions of morphine (● 10 μg/hr) or saline (○ 1 μl/hr). Data are presented as the mean±S.E.M. of 3–4 animals. The dose-response curves represent the results of two separate experiments. In one experiment, 10 and 15 mg/kg morphine were injected on days 4 and 5 of continuous intrathecal infusion, respectively. In the other experiment, 2, 4 and 8 mg/kg morphine were injected on days 5, 6 and 7 of continuous infusion, respectively.

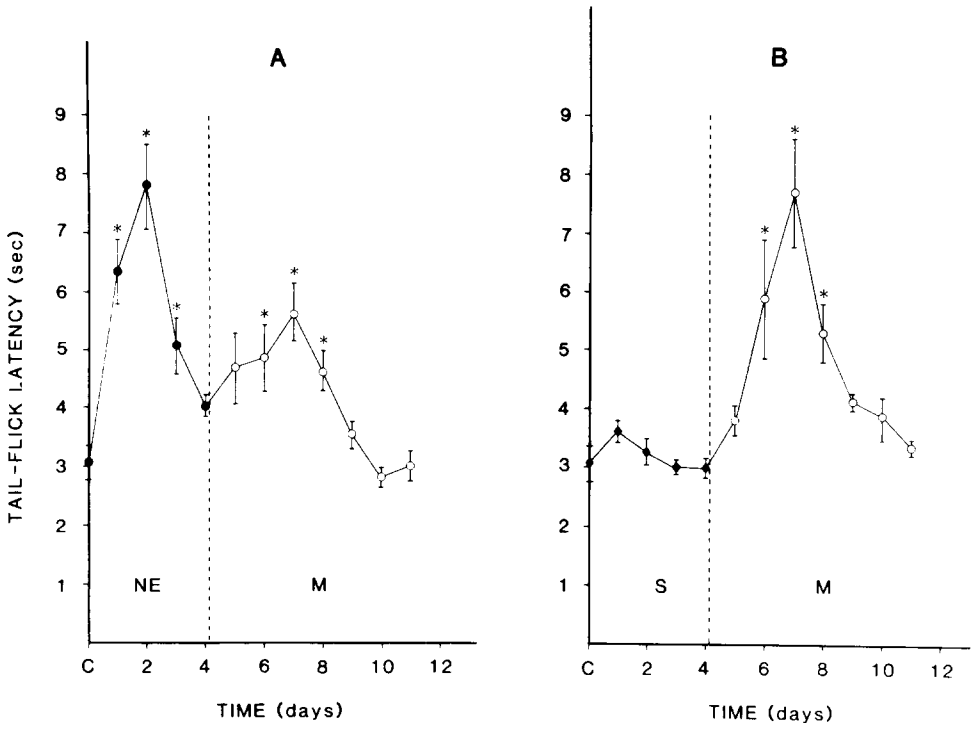

FIG. 5. The effect of continuous intrathecal infusion of: A: norepinephrine (NE) (● 15 μg/hr) for 4 days followed by morphine (M) (○ 10 μg/hr) for 7 days on tail-flick latency. B: saline (S) (◆ 1 μl/hr) for 4 days followed by morphine (M) (○ 10 μg/hr) for 7 days on tail-flick latency. Data are expressed as the mean±S.E.M. of 6 and 7 rats for group A and B, respectively. The * denotes significant difference from control (C).

continuous IT infusion as well as repeated exposure to halothane and surgery did not adversely affect the animal, body weight, motor function and behaviour were assessed daily throughout the experiment.

Data Analysis

Statistical comparison of the tail-flick latencies within each group was conducted using one-way, repeated measures analysis of variance followed by Newman-Keuls tests for a significant F ratio. Statistical comparison of the analgesic effect of each IP dose of morphine in continuous IT morphine- and saline-treated rats was conducted using the unpaired Student's *t*-test. For IT morphine infusion in the double pump study, statistical comparison of the tail-flick latencies in NE- and saline-pretreated rats at the individual time points was conducted using the unpaired Student's *t*-test. The area-under-curve (AUC) value for each animal during continuous IT morphine infusion in the double study was determined using a trapezoidal integration program for discrete points. Comparison of the mean AUC values for continuous IT morphine in NE- and saline-pretreated rats was conducted using the unpaired Student's *t*-test. Two sets of data were defined as significantly different when statistical analysis indicated $p<0.05$.

RESULTS

Simple Pump Experiment

The rostal and caudal migration of methylene blue at various times during continuous IT infusion are shown in Table 1. There was a progressive but limited movement of dye from the tip of the lumbar catheter with time. The extent of migration ranged from 0.25 cm on either side of the catheter tip after 24 hr to 1.5 cm rostrally and 0.5–1.0 cm caudally after 5–7 days of infusion. There was very intense staining of the spinal tissue with this dye and it was easy to identify the margin of dye migration and the tissue sites which had been stained. The data in Table 1 are the distances from the tip of the catheter at which any tissue staining was observed. However, visual examination of the spinal cord showed that the most intense staining occurred around the tip of the catheter for a total rostral-caudal distance of 1.5 cm. There was no evidence of cervical or supraspinal staining after 7 days of continuous infusion. The SC minipump was examined in each animal to ensure that the dye had not leaked from the connection between the pump and the PE 60 tubing or from the the connection between the PE 10 and PE 60 tubing. No leaks were observed at any of these sites. These data show that, even with a hydrophilic dye such as methylene blue, the slow microinfusion rate of the miniosmotic pump does not yield extensive redistribution along the neuraxis of the rat after 7 days of infusion. [The n-octanol/water partition coefficient of methylene blue is 0.15±0.006 (mean±SD of five determinations). Methylene blue (250 μg) was added to a mixture of 10 ml n-octanol and 10 ml of distilled water (pH 6.8) and shaken for 30 min at 22°C with an Eberbach reciprocal shaker. The concentration in each phase was determined by spectrophotometric analysis (λ max. 608 nm).]

Figure 2 shows the time course of tail-flick latency during continuous IT infusion of morphine (10 μg/hr) or saline (1 μl/hr) for rats given the 10 and 15 mg/kg doses of IP morphine. Maximum analgesia was observed on day 3 of morphine infusion. The delay in the onset of analgesia is due to the dead space of the catheter and reflects the time required for the drug to reach the IT space and for the minipump to achieve a stable output (approx. 6 hr; Alza Technical Bulletin). Tolerance was apparent by day 4 and continued to progress for the duration of infusion. The development of tolerance to the antinociceptive effect of morphine during continuous IT infusion is similar to that reported for morphine using repeated, daily IT injections [39], as well as previous studies using continuous IT infusion [17, 26, 31]. There was no significant increase in tail-flick latency during the continuous IT infusion of saline.

The time course of analgesia following the IP administration of 10 and 15 mg/kg morphine to rats receiving continuous IT morphine or saline are shown in Fig. 3. The 10 and 15 mg/kg doses of morphine were given on days 4 and 5 of continuous IT infusion, respectively. All points were significantly different from their respective controls except for 180 min in the saline group given 10 mg/kg morphine. Maximum analgesia, equivalent to the 10 sec cutoff, was observed at 30 to 45 min. For 10 mg/kg morphine, the response latency in the IT morphine-treated group was significantly greater than the IT saline-treated group at control, 150 and 180 min. For the 15 mg/kg dose, only the control response latency was significantly greater in the IT morphine-treated group as compared with the saline-treated group. These significant differences reflect the additional antinociceptive effect of IT morphine. As shown in Fig. 2, tail-flick latency had not returned to baseline on days 4 and 5 when the effects of systemic morphine were tested. Complete tolerance to the spinal effect of morphine did not occur until day 6 of infusion. There was no attenuation of the antinociceptive effect of systemic morphine in animals tolerant to IT morphine. Similar results were observed in the tail-flick latency vs. time, and paw pressure threshold vs. time curves during continuous IT morphine and saline infusions for the second group of rats (e.g., rats given 2, 4 and 8 mg/kg morphine IP) (data not shown).

The dose-response curves for IP morphine in rats receiving either continuous IT morphine or saline are shown in Fig. 4. These curves were constructed from the time course data for all doses of IP morphine using the maximum response for each dose. In IT morphine- and saline-treated rats, systemic morphine produced a dose-dependent and almost equal increase in the tail-flick latency and the paw pressure threshold. There were no significant differences in the effect of systemic morphine between the saline- and morphine-treated groups except for the 2-mg/kg dose in the tail-flick test. These data indicate that rats, receiving a continuous IT infusion of morphine for up to 7 days, remain responsive to the effects of systemic morphine despite the presence of spinal tolerance. The fact that their response was comparable to the effect observed in animals given continuous IT saline indicates that supraspinal sites, and possibly spinal sites not reached by the chronic infusion, mediating morphine antinociception remained unaffected. These data are in agreement with the methylene blue experiments and suggest that there was little redistribution of morphine from the lumbar site of infusion to higher sites either through bulk CSF flow or via the blood circulation.

In a separate group of animals, the ability of IP naloxone to precipitate withdrawal symptoms in rats receiving morphine by continuous IT infusion (10 μg/hr) was determined. Tail-flick latency was used to assess the analgesic effect of spinal morphine. Immediately after testing on day 3 of morphine infusion, naloxone (1 mg/kg) was given by IP injection and the animals were monitored for signs of withdrawal. Naloxone produced characteristic symptoms of withdrawal in all animals including irritability, vocalization and defecation. Furthermore, IP naloxone temporarily antagonized the antinociceptive effect of spinal morphine (data not shown).

Double Pump Experiments

Figure 5 shows the time course of tail-flick latency during the continuous IT infusion of norepinephrine (NE; 15 μg/hr) or saline (1 μl/hr) for 4 days, followed by morphine (10 μg/hr) for 7 days. For NE infusion, the tail-flick latency was significantly greater than control at all time points with the maximum antinociceptive effect occurring on day 2. Tolerance, as indicated by a decline in antinociception during IT infusion, was apparent by day 3 and continued thereafter. There was no significant increase in tail-flick latency at any time during the continuous IT infusion of saline. In NE-tolerant animals, the continuous infusion of morphine produced a significant increase in tail-flick latency on days 6, 7 and 8 (days 2, 3 and 4 of morphine infusion) as compared to control. The maximum increase in tail-flick latency (5.7 sec) occurred on day 3 of morphine infusion. Tolerance was apparent by day 4 and tail-flick latency returned to control on day 5. In saline-treated rats, continuous IT infusion of morphine significantly increased tail-flick latency on days 6, 7 and 8 (days 2, 3, and 4 of morphine). The maximum increase in tail-flick latency (7.7 sec) occurred on day 3 of morphine infusion. Comparison of mean tail-flick latencies during IT morphine infusion in saline- and NE-pretreated rats at individual time points indicated significant differences on days 9 and 10 only (days 5 and 6 of morphine infusion). When the data for morphine infusion in the two groups were analyzed using area-under-curve (AUC) values, no significant difference was determined. The AUC for morphine in saline-pretreated rats was 30.4±2.50 (mean±SD) and 26.2±1.98 in the NE-pretreated group. The data indicate that spinal tolerance to NE did not significantly affect the analgesic action of IT morphine in rats as assessed by the tail-flick test.

It is important to note that tolerance to NE during continuous IT infusion was not due to oxidation of NE in the mini-osmotic pump. The concentration of NE in the residual solution of pumps removed from NE-treated rats was determined using fluorometric analysis as previously described [17]. Pumps were removed on day 4, and 50 μl of solution were withdrawn from each pump using a Hamilton microsyringe. There were no decreases in NE concentration after 4 days of infusion (15 μg/μl).

DISCUSSION

The development of the technique of chronic catheterization of the spinal cord in the rat [38], and the identification of opioid receptors in the spinal cord, led to an understanding that opioid analgesia is mediated, in part, in the spinal cord [32,33]. The first studies of spinal opioid tolerance were conducted using repeated IT injection [39]. These studies confirmed that tolerance does develop to the analgesic effects of opioids in the spinal cord. Repeated IT injections were also used to study the extent of cross

tolerance to other opioid and non-opioid analgesics [36]. When a drug is administered by repeated injection on a scheduled basis, there are large and frequent fluctuations in the concentration of the drug at the site of injection. Furthermore, tests for tolerance and cross tolerance are often conducted at the time of the next scheduled injection when the concentration of the original drug is decreasing and may be low. This has been shown in previous investigations to affect the determination of tolerance and cross tolerance with opioids [13,14]. Recently, continuous IT infusion using mini-osmotic pumps has been used to study the chronic effects of opioids and to induce tolerance in the spinal cord of the rat [17, 26, 27, 31]. In two studies of cross tolerance between morphine and D-Ala^2-D-Leu^5-Enkephalin, tolerance was initially induced in the spinal cord using continuous IT infusion [26,27]. The minipump was then removed under anesthesia and the IT catheter was cleared of drug solution by flushing the remaining drug into the spinal cord. Cumulative dose-response curves were determined 2 or 4 hr later using repeated IT injections. In contrast to previous studies using repeated IT injection, cross tolerance in Tseng's experiments [26,27] was determined when the concentration of the original drug was probably still high in the spinal cord. However, this procedure required the infusion of large volumes of drug solution and vehicle into the subarachnoid space of the rat over a relatively short period of time. This can have an important effect on the experimental results for two reasons. First, the volume of cerebrospinal fluid in the spinal cord of the rat is naturally small. The IT injection of even μl-amounts of solution can therefore increase CSF pressure, which in turn, can produce secondary effects and nonspecific antinociception. Second, the redistribution of hydrophilic drugs like morphine from the spinal site of injection to supraspinal sites is primarily due to bulk CSF flow within the spinal cord. This redistribution is known to be influenced by the volume of solution injected IT [20,24]. Therefore, it is difficult to know if the effects observed under these conditions are exclusively spinal in origin or if they also represent the involvement of more rostral components known to be involved in the processing of nociceptive information.

The slow microinfusion rates of mini-osmotic pumps (0.5–1.0 μl/hr) make them particularly useful for continuous IT infusion in the rat. In comparison with repeated IT injection, this technique provides even delivery of the drug over time thereby preventing large fluctuations in drug concentration in the spinal cord, requires less animal handling during the experiment, is much more convenient for chronic drug administration, and provides a very slow and precise infusion rate. In this report, we have described the use of two mini-osmotic pumps in sequence for the investigation of cross tolerance in the rat spinal cord. Before using this double pump technique, it was important to: (1) assess the reliability of continuous IT infusion via mini-osmotic pumps in delivering drug to the spinal cord of the rat, (2) to determine if the effects observed during infusion were spinal effects, and (3) to establish that tolerance and physical dependence to morphine could be produced with this infusion system.

In assessing the reliability of continuous IT infusion for tolerance and cross tolerance studies in the rat, several questions were considered. First, is the catheter in the subarachnoid space of the spinal cord and does it remain in the rostral segment of the lumbar enlargement throughout the implantation and removal of the mini-osmotic pumps? To answer this question, animals were randomly selected from all treatment groups at the end of our double pump experiments and given a lethal dose of urethane by IP injection. The pumps were disconnected from the IT catheter and injections of methylene blue dye were made through the catheter with a hand held syringe. Laminectomy was performed to expose the dorsal surface of the spinal cord and the tip of the catheter was located. This procedure confirmed both the patency and position of the catheter in the lumbar subarachnoid space of all animals tested. In addition, it verified that there were no leaks in the connections between the pump and tip of the catheter through which the drug solution could escape before reaching the spinal cord.

The extent of drug migration from the site of infusion in the lumbar spinal cord during continuous IT infusion was assessed by determining the rostral and caudal migration of methylene blue dye in the spinal cord at various times during infusion. Even after 7 days of continuous IT infusion, the dye remained localized near the tip of the IT catheter. For opiate analgesics, the problem of rostral migration and delayed respiratory depression following acute spinal administration is greatest for hydrophilic opiates like morphine (see review [18]). Since methylene blue (octanol/water partition coefficient of 0.15 at 22°, pH 6.8) is more hydrophilic than morphine (1.17 at 20°, pH 7.4 [12]), methylene blue might be expected to undergo more extensive rostral/caudal redistribution than morphine. However, methylene blue is a dye with an affinity for protein which could limit the extent of redistribution during IT infusion. Currently, we are investigating the distribution of radiolabelled morphine in the spinal cord and the brain during continuous IT infusion.

If morphine remains localized in the spinal cord during continuous IT infusion, no tolerance should develop to the supraspinal actions of morphine following IP administration. With the exception of the lowest dose of systemic morphine, there were no significant differences in the dose-response curve for IP morphine in rats receiving continuous IT infusions of morphine or saline. These data provide indirect evidence that morphine remains localized in the spinal cord using a 1 μl/hr infusion. It would appear that this infusion rate is sufficiently slow to prevent extensive redistribution from the site of injection via bulk CSF flow in the spinal cord or blood circulation. These results demonstrate the usefulness of this technique for determining the chronic effects of drugs at the spinal level. They also demonstrate that it is possible to produce significant, dose-dependent analgesia with systemic morphine in the presence of spinal tolerance. The absence of saline-induced analgesic effects during continuous IT infusion indicates that non-specific antinociception is not a problem with this technique.

Previous studies using chronic systemic morphine administration have shown that the spinal cord is involved in the development of tolerance and physical dependence to morphine [3, 7, 8, 16]. To confirm that continuous IT morphine infusion produces tolerance and physical dependence in the rat, the ability of naloxone to precipitate withdrawal symptoms was determined. The results of this experiment clearly showed that some of the withdrawal symptoms observed in studies using chronic systemic morphine administration could be precipitated by naloxone during continuous IT morphine infusion. In addition, the effect of IT morphine on tail-flick latency was antagonized by naloxone in this experiment, indicating that the analgesia observed during morphine infusion was mediated by mu receptors.

In a previous investigation using sequential double mini-osmotic pumps, NE-induced spinal analgesia was signifi-

cantly attenuated in rats tolerant to spinal morphine [17]. In the present study, this technique was used to determine if rats, tolerant to spinal NE, demonstrated cross tolerance to IT morphine. Significant analgesia was achieved with continuous IT morphine after tolerance had developed to IT NE. Tolerance to spinal NE did not significantly affect the analgesic action of morphine. Furthermore, there was no difference in the development of tolerance to morphine (e.g., rate and magnitude) between saline- and NE-pretreated rats. These data suggest that morphine, injected into the spinal cord of the rat, does not produce spinal analgesia by activation of local adrenergic systems. If this were true, it is expected that morphine-induced analgesia would be significantly reduced by tolerance to NE. In this regard, it was recently reported that the increase in the paw pressure threshold produced by the acute IT injection of 2 or 10 μg morphine sulfate was not significantly affected by 90% depletion of spinal cord NE using 6-hydroxydopamine [19].

The absence of cross tolerance to NE in the present study may be due, in part, to the recovery of adrenergic receptor sensitivity during morphine administration. This is because the continuous IT infusion of NE was discontinued at the time of morphine infusion. The time course of recovery of spinal adrenergic receptors for NE following continuous IT infusion is not known. If the rate of recovery of sensitivity to NE following continuous IT infusion is similar to the rate of tolerance development (e.g., 3–4 days), then maximum analgesia with IT morphine occurred well before recovery was complete. In the rat, recovery of sensitivity to IT morphine after cessation of repeated IT morphine injection was reported to be incomplete after 7 days [28].

Behavioural withdrawal symptoms and losses in body weight were not observed in either this study or the previous investigation [17], when continuous IT infusion was discontinued. The development of withdrawal is important because of previous reports describing the effect of opioid withdrawal on both tolerance and cross tolerance to systemic and ICV opiates (e.g., withdrawal tolerance) [13,14]. The question of withdrawal tolerance would not appear to be important in the present study since spinal tolerance to NE did not significantly affect IT morphine analgesia. However, the data indicate that morphine-induced analgesia was slightly reduced in NE- as compared with saline-pretreated rats. It is possible that the observed difference in morphine AUC between the two groups represents some degree of withdrawal tolerance following cessation of continuous IT NE.

Failure to observe withdrawal in our studies may be due to several factors. The withdrawal symptoms could be very subtle and therefore difficult to detect and/or they may have occurred at a time when the animals were not being monitored. Alternatively, IT NE may have suppressed the opiate withdrawal symptoms in our previous study by mimicking the action of clonidine on $\alpha 2$ receptors in the spinal cord. Intrathecal clonidine has been shown to prevent the hyperalgesia associated with opiate withdrawal in the rat [16]. In the present study, IT morphine may have suppressed NE withdrawal. These questions are currently being investigated in our laboratory.

In summary, continuous IT infusion via mini-osmotic pumps appears to be very useful for studying spinal tolerance and cross tolerance in the rat. The slow infusion rate limits the redistribution of drug in the spinal cord, minimizing drug effects at supraspinal sites. Anesthesia, surgery, and the presence of both the IT catheter and the mini-osmotic pump are well tolerated by the animals. Generally, there is little or no loss in body weight during infusion and the development of hindlimb weakness or paralysis is very rare when low doses of drugs are used. The IT catheter remains internalized throughout infusion and therefore cannot be pulled out or displaced by the rat. Studies are now in progress to determine the extent of cross tolerance between opioid receptor selective agonists and monoamines using this double pump technique.

ACKNOWLEDGEMENTS

The technical assistance of Ms. M. Sutak is gratefully acknowledged. The authors would also like to thank Dr. Khem Jhamandas for his suggestions and discussions of this work.

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