Epidural Injections of Bupivacaine, Morphine, Fentanyl, Lofentanil, and DADL in Chronically Implanted Rats: A Pharmacologic and Pathologic Study

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A new technique of epidural catheterization in rats is described. The pharmacologic characterizations of the model were established after epidural injection of bupivacaine, morphine, fentanyl, lofentanil, and D-Ala²-D-Leu²-enkephalin (DADL) on hot plate (HP) and tail flick (TF). In addition, rostral spread, motor function, behavior, and reproducibility of the effects over time were assessed. The time-response curves showed an almost immediate onset of action for bupivacaine, fentanyl, and lofentanil and a delayed onset for morphine and DADL. Morphine and lofentanil displayed a significantly longer duration of action than bupivacaine, fentanyl, and DADL. The dose–response curves were monotonic and the slopes were log-linear. Based on the ED₅₀ values, the following rank order of potency was obtained 1 day after catheterization for both HP and TF: lofentanil > fentanyl > morphine > DADL > bupivacaine. Intraperitoneal (IP) administration of naloxone antagonizes the agonist effects of epidural morphine, fentanyl, and lofentanil. To assess the role in analgesia played by epidural vascular uptake after epidural administration of morphine, fentanyl, and lofentanil, the lowest maximally effective epidural dose of these agents was given intravenously. After iv fentanyl and lofentanil, the analgesic and behavioral effects were not different from the values obtained after epidural administration. By contrast, the effects were negligible after iv morphine when compared with the epidural route. Epidural vascular uptake is thought to be low for morphine and high for fentanyl and lofentanil. The reproducibility of the analgesic and behavioral effects over time was assessed by epidurally injecting the lowest maximally effective dose of bupivacaine, morphine, fentanyl, and lofentanil 1 day and 10 days after catheterization. After 10 days, a significant reduction of analgesic and behavioral effects was noted and was thought to be due to a complete fibrotic sheath surrounding the epidural catheter. (Key words: Anesthetic techniques: epidural narcotic. Analgesics, narcotic: morphine; fentanyl; lofentanil; tolerance. Anesthetics, local: bupivacaine. Antagonists, narcotic: naloxone.)

The observations that opiates with an action limited to the spinal cord will inhibit nociceptive reflexes and that the intrathecal (IT) injection of opiates will produce analgesia in a variety of animal models have led to spinal administration of narcotics for pain relief in humans. The study of the spinal pharmacology and toxicology was facilitated by the early development of a chronic IT animal model. Recent efforts have been directed at developing a comparably simple epidural model to allow investigation of the characteristics of drugs administered by the epidural route, an approach that is commonly employed clinically. In 1981, Van den Hoogen and Colpaert described a method for chronically catheterizing the lumbar epidural space of the rat. In this technique, part of the spinal process and arch of the third lumbar vertebra L₃ are removed with an electric dental burr. In skilled hands, the whole procedure takes about 35 min; the animals are given at least 1 week to recover before testing. In 1984, Bahar et al. used the same technique for both intrathecal and epidural cannulation, the dura being torn gently for the former and left unopened for the latter. In these two studies, the success rate of epidural catheterization is not mentioned and the reproducibility of the technique for chronic evaluation of analgesia is assumed, although no data comparing drug response over time are shown.

This article describes a simple technique of lumbar epidural catheterization in rats. The characteristics of the model (e.g., rostral spread, analgesia, motor function, behavior, and reproducibility of the drug effect over time) are assessed in awake animals after lumbar epidural injection of a variety of opioid and nonopioid agents.

Materials and Methods

Preparation of Epidural Catheters

To make the catheters, polyethylene tubings (PE-10, 0.28 mm inside and 0.61 mm outside diameters) were cut into segments 17 cm long. A loose overhand knot about 2 cm from one end was fixed with cranioplast cement (fig. 1A). Before implantation, each catheter was cleaned with acetone for several minutes and then rinsed and flushed with sterile saline.

Animal Preparation and Surgical Procedure

Anesthesia was induced in male Sprague-Dawley rats (250–300 g) (Harlan Industries, Indianapolis, Indiana) with 4% halothane in air administered for 2 min with a plastic mask and maintained with 2% halothane until the end of the procedure. The skin of the back was depilated from the tail to the neck and tincture of Merthiolate® applied. To flex the lumbar vertebral column during surgery, a 60 ml syringe was placed transversely under the animal’s abdomen. The crests of the ilium were palpated

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to locate the sixth lumbar vertebra L-6, which lies between them. A midline skin incision was made over the spinous processes of the L-5 and L-6 vertebrae.

The fascia was opened and the ligamenta interspinalis between the spinous processes of L-5–L-6 and L-6–S-1 was cut. The superficial muscles around the spinous process of L-6 were dissected and retracted. The L-6 spinous process was removed to allow the insertion of the catheter parallel to the dura. A dissecting microscope then was used at ×10 magnification. The area between the two posterior articular processes of L-5 was cleared to reach the yellow ligament. To increase the distance between the dura and the yellow ligament, the curve of the lumbar spine was maximized by the operator. Using a right-angle hook made of a 22-gauge needle (fig. 1A), the yellow ligament was pierced at the base of the L-5 spinous process.

The catheter gently was introduced cephalad into the lumbar epidural space to a length of about 2 cm, the piece of cranioplast cement occupying the place of the removed L-6 spinous process (fig. 1C). The catheter tip was now located at the L-3 level, one segment above the caudal end of the spinal cord. The catheter was flushed with sterile saline, and no solution would leak around it for volumes up to 100 µl. As shown in figures 1B and C, it is not necessary to burr a groove in the vertebral arch to reach the lumbar epidural space.

Two stitches were put between the superficial muscles above the cranioplast cement to avoid movement of the
catheter. The remainder of the catheter was tunneled subcutaneously, 3 cm emerging through the skin in the neck region. The skin incision was closed with several stitches. The entire procedure required approximately 10 min. After discontinuation of halothane, all animals showed normal motor function within 10 min.

At other times, the anatomy of the caudal epidural space was examined carefully in several animals. The caudal end of the dura was located under a microscope at the S-3, S-4, and Co-1 levels in 4, 1, and 2 rats, respectively. The caudal end of the epidural space was located at the S-4, Co-1, Co-2 and Co-3 levels in 3, 3, 2, and 1 rats, respectively. At the sacral and coccygeal levels, the epidural space was very narrow and filled with caudal nerves (fig. 1D). Therefore, in contrast to our personal observations in dogs and cats, the coccygeal approach to the epidural space is not to be recommended.

**NOCICEPTIVE TESTS**

The effects of epidural agents were examined on paw-pinch stimulation (PP) and on thermal tests: hot plate (HP) and tail flick (TF).

*Paw-Pinch.* The forepaws and hindpaws were pinched with a Kocher forceps. The withdrawal response was assessed and considered as positive or negative.

*Tail Flick.* The TF response, polysynaptic spinal reflex, was thermally evoked by laying the tail of the rat over a slit, through which the light of a 300 W quartz bulb was focused. Time between stimulus presentation and the rapid removal of the tail from the slit was defined as the response latency. To prevent tissue damage, the trials were terminated after 6 s.

*Hot Plate.* The HP response was evaluated by placing the animal on a metal surface maintained at 52.5°C. The response latency was defined as the time between placing the animal on the surface and the licking of one or the other hindpaw. In a few animals, jumping was observed, and this escape behavior was considered the end point of the test. Cut-off time in the absence of a response was 60 s.

**NONOCICEPTIVE TESTS**

*Motor Function.* The ability to negotiate a 60-degree inclined plane was assessed. The rat was placed facing upgrade on a 60-degree inclined wire mesh surface. Untreated rats showed facile reversal of direction and coordinated descent. Impairment in negotiating the inclined plane was defined as motor dysfunction. The presence of truncal (“banana” rat) and tail rigidity (“straub” tail) also were noted.

*Behavioral Observations.* Catalepsy was defined as lack of movement for a period of greater than 15 s when the animal’s forelimbs were placed on a bar 4 cm above the floor. Animals also were observed carefully for scratching and abdominal breathing.

**DRUGS AND THEIR INJECTION**

To assess the epidural volume producing sensory and motor blockade of the lower half of the body, 10 μl (n = 5), 20 μl (n = 5), and 40 μl (n = 5) of bupivacaine 0.75% were injected epidurally. Sensory and motor blockade of the hindpaws (block of agitation to pinch) but normal sensory and motor functions of the forepaws and maximal nociceptive response latencies on HP and TF were observed with 40-μl volumes. Based on these preliminary data, drugs for epidural injections were mixed such that all doses were administered in a 40-μl solution followed by a 10-μl 0.9% sodium chloride vehicle to flush the catheter (“dead space” of the catheter = 10 μl). After flushing, the tip of the catheter was closed with a stainless steel pin.

Drugs administered epidurally (EPI) were as follows: bupivacaine hydrochloride (Sensorcaine®), morphine sulfate, fentanyl citrate (Sublimaze®), lofentanil oxalate, and D-Ala²,D-Leu⁵-Enkephalin (DADL). Bupivacaine, morphine, and fentanyl were chosen because of their common clinical use. Lofentanil was chosen for its extremely potent and long-acting analgesic properties.⁴ DADL was chosen to compare an opioid peptide with the opiate drugs. Moreover, intrathecally administered DADL has significant analgesic properties in rats, primates, and humans.⁵-⁷ All animals that received epidural injections were injected and tested 24 h after surgery. All the drugs were diluted with saline. Because of its high lipophilicity, lofentanil was dissolved in dimethylsulfoxide (20%) (DMSO) and saline (80%) to achieve the desired concentration.

Regarding the initial ranging of dosage, an “up-and-down” method was used. Briefly, the highest dose shown to have some effect after intrathecal injection was administered epidurally. Failure to observe significant analgesia on the HP and TF tests at that dose dictated a threefold increase in the dose administered to the next rat. Conversely, a significant effect dictated that one-third of the dose was given to the subsequent rat. In this fashion, it was possible to determine in a few animals the lowest maximally effective epidural dose: lofentanil 0.6 nmol (0.3 μg), fentanyl 5.7 nmol (5 μg), morphine 30 nmol (10 μg), and bupivacaine 930 nmol (300 μg).

To assess opiate antagonism, three groups of animals received 1 mg/kg naloxone intraperitoneally (ip), 5 min before the lowest maximally effective epidural dose of lofentanil (n = 3), fentanyl (n = 3), or morphine (n = 4). A fourth group (n = 4) received 10 mg/kg naloxone ip 5 min before the epidural injection of lofentanil 0.6 nmol.
Drugs for ip injection were given in a volume of 0.1 ml of solution per 100 g of body weight. We sought to determine if the drug effects of the lipid-soluble agents might result from systemic redistribution. The lowest maximally effective epidural dose of morphine, fentanyl, and lofentanil was given iv in three different groups of animals (n = 4, in each group). Drugs for iv injections were administered in a 40-μl solution in the femoral vein. DADL and bupivacaine were not administered intravenously.

To assess the reproducibility of the analgesic and behavioral effects over time, the groups that received epidurally the lowest maximally effective dose of lofentanil, fentanyl, morphine, or bupivacaine were injected and tested 24 h after surgery (EPI 1) then injected and tested again 10 days after surgery (EPI 10).

**PATHOLOGY**

After completion of the experimental series, the vertebral column of each animal was removed and a lumbar laminectomy was performed with the use of a dissecting microscope to check the position of the catheter and to study the nature of the local reaction around the catheter.

For histologic observations, three rats had catheters implanted and received no drugs. They were killed 1 day, 2 days, and 10 days after surgery, respectively. The vertebral columns were removed, fixed in formalin for 7 days, decalcified in 10% formic acid, and embedded in paraffin. The sections were cut at 6 μm and stained with hematoxylin and eosin (H and E).

**STATISTICAL ANALYSIS**

Response latencies were expressed as the mean ± SE or as the per cent of the maximum possible effect (MPE) ± SE:

\[
\%\text{MPE} = \frac{\text{postdrug latency} - \text{predrug latency}}{\text{cut-off time} - \text{predrug latency}} \times 100\%
\]

The areas under the time–response curves (AUC) were expressed in MPE × minutes and were measured by the "trapezoidal rule." Briefly, each AUC is the sum of the individual trapezoids in which the heights are the response latencies minus the predrug baseline latencies and the bases, the time intervals between measurements. To compare the EPI 1 group versus the EPI 10 group, a paired t test was used. To compare the EPI 1 group versus the iv group, an unpaired t test was used.

A least-squares regression analysis was used to calculate the ED₉₀ values, 90% confidence intervals, and correlation coefficients of the dose–response curves.

The antagonism by naltrexone was expressed as per cent MPE. The drug group and drug + naltrexone group were compared with the t test. When more than two comparisons were to be made, one-way analysis of variance was employed with a Newman-Keuls multiple means analysis.

**Results**

**SURGICAL PROCEDURE**

A total of 118 animals were prepared. Of these animals 105 (89%) were found to have the catheter in the epidural space; 12 (10.2%) catheters were in the intrathecal space; and 1 was (0.8%) in the lateral foramen (as defined by the presence of methylene blue). The key factors in obtaining a high success rate were the use of a right angle hook and the flexion of the lumbar spine during perforation of the yellow ligament. During necropsy, we observed that maximal flexion increased the yellow ligament–dura distance up to 1 mm at the L-5 level. At the end of each necropsy, the catheter was removed and flushed. None showed signs of leaking.

**EFFECT OF EPIDURAL DRUGS ON TAIL FLICK AND HOT PLATE RESPONSE LATENCY AND ON PINCHING**

Figure 2 presents the time course of the effect of bupivacaine, morphine, fentanyl, lofentanil, DADL, and saline on the TF and HP responses, 24 h after catheteriza-
TABLE 1. Summary of Behavioral Signs Observed after Intravenous or Epidural (1 day of catheterization = EPI 1; 10 days = EPI 10) Injection of Bupivacaine, Morphine, Fentanyl, Lofentanil, and DADL in the Rat

<table>
<thead>
<tr>
<th></th>
<th>Dose (nmol)/40 μl</th>
<th>Motor Dysfunction*</th>
<th>Absence of Withdrawal during Pinching</th>
<th>Time to Onset (min)</th>
<th>Time to Regain Normal (min)</th>
<th>Truncal and/or Tail Rigidity</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Hindpaw</td>
<td>Forepaw</td>
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<tr>
<td>Bupivacaine</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>EPI 1</td>
<td>155</td>
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<td>4</td>
<td>—</td>
<td>—</td>
<td>&lt;1</td>
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<tr>
<td></td>
<td>310</td>
<td>3/3</td>
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<td>&lt;1</td>
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<tr>
<td></td>
<td>930</td>
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<td>5</td>
<td>—</td>
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<td>&lt;1</td>
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<tr>
<td>EPI 10</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td></td>
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<td></td>
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<tr>
<td>EPI 1</td>
<td>3-9</td>
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<td>—</td>
<td>—</td>
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<td>—</td>
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<td></td>
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<tr>
<td>EPI 10</td>
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<td>0/9</td>
<td>—</td>
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<td>—</td>
<td>—</td>
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<tr>
<td>iv</td>
<td>30</td>
<td>0/4</td>
<td>—</td>
<td>—</td>
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<td>Fentanyl</td>
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<tr>
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<td>1/5</td>
<td>1</td>
<td>—</td>
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<td>&lt;1</td>
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<tr>
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<td>3/4</td>
<td>3</td>
<td>3</td>
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<td>—</td>
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<td>—</td>
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<tr>
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<td>3/4</td>
<td>—</td>
<td>2</td>
<td>&lt;1-15</td>
<td>&lt;1-15</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>4/4</td>
<td>4</td>
<td>4</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>EPI 10</td>
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<td>1/4</td>
<td>—</td>
<td>2</td>
<td>&lt;1</td>
<td>&lt;1-60</td>
</tr>
<tr>
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<td>4/4</td>
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<td>4</td>
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<td>&lt;1</td>
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<td>0/13</td>
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</table>

* Number of animals showing dysfunction/number of animals injected.

Almost, unlike morphine, the opioid peptide and putative delta ligand, DADL, did not produce a complete block of the TF and HP responses at the highest dose used in the dose range examined: 12.3-140.4 nmol (7-80 μg). Therefore, DADL was not tested 10 days after catheterization. Fentanyl on the HP and bupivacaine on the TF produced a submaximal elevation at the highest doses, 5.7 nmol and 950 nmol, respectively. After epidural injection of bupivacaine (950 nmol), the TF response was hyperreflexive. Among the drugs producing a complete block, only morphine had a delayed peak effect at 15 min on both TF and HP. No significant effect was noted after epidural injection of saline.

As shown in table 1, after 1 day of catheterization, at the dosages producing a maximal or submaximal responses on HP and TF, no withdrawal was noted for bupivacaine (five out of five) during pinching of the hindpaws and for fentanyl (four out of five) and lofentanil (four out of four) during pinching of both forepaws and hindpaws. Withdrawal was noted for morphine during pinching of both forepaws and hindpaws. In contrast, 10 days after catheterization, vigorous withdrawal was observed in bupivacaine-, fentanyl-, and lofentanil-treated animals.

Considering the areas under the time-response curves (AUC) as an index of the duration and intensity of drug action, table 2 shows the following rank order after 1 day of catheterization for HP: lofentanil > morphine > bupivacaine > fentanyl > DADL. For the TF, the following rank order was obtained: morphine = lofentanil > bupivacaine = fentanyl > DADL. After 10 days of catheterization, the AUCs were significantly reduced for morphine and fentanyl on both HP and TF and for lofentanil on HP. Similarly, the %MPE values were significantly reduced, except for bupivacaine on TF and lofentanil on both HP and TF.

**Effect of Intravenous Drugs on TF and HP Response Latency and on the Pinch Withdrawal Response**

Tables 1 and 2 show normal withdrawal of the paws on PP and no effect on HP and TF response latencies after iv morphine. However, significant effects were produced by iv fentanyl and lofentanil on all measures whether assessed in terms of the %MPE or the AUC.
RANK ORDER OF EPIDURAL POTENCY

As shown in figure 3, the five drugs injected epidurally after 1 day of catheterization display positive monotonic dose–response curves over the range of the measurement. Table 3 shows that most of the slopes were statistically greater than zero. Table 3 also shows the 90% confidence intervals of the slope, the correlation coefficient, and the ED50 values with 90% confidence intervals expressed in nanomoles for the effects of epidurally administered agents after 1 day of catheterization on the TF and HP. Based on the ED50 values, the rank order of potency for both TF and HP is as follows: lofenatanil > fentanyl > morphine > DADL > bupivacaine.

EFFECT OF NALOXONE ON THE AGONIST EFFECTS OF EPIDURAL MORPHINE, FENTANYL, AND LOFENATANIL AFTER ONE DAY OF CATHETERIZATION

The ip administration of naloxone, 1 mg/kg, 5 min before the epidural injection of morphine 30 nmol and fentanyl 5.7 nmol resulted in significant antagonism as judged by the %MPE values for both HP and TF. Lofenatanil 0.6 nmol was significantly antagonized by naloxone 1 mg/kg for HP and by naloxone 10 mg/kg for both HP and TF (fig. 4).

BEHAVIORAL OBSERVATIONS

Behavioral dysfunction frequently was observed at doses producing a maximal effect on HP and TF, when administered 1 day after catheterization. In contrast, 10 days after catheterization, the behavioral effects were far fewer than after 1 day (table 1).

After 1 day of catheterization the epidural injection of bupivacaine (310 nmol, 930 nmol), morphine (30 nmol, 90 nmol), fentanyl (5.7 nmol), and lofenatanil (all doses) and the intravenous injection of fentanyl (5.7 nmol) and lofenatanil (0.6 nmol) had a significant effect on coordinated motor function (ability to negotiate a 60-degree inclined plane). After 10 days of epidural catheterization, one rat out of five that received fentanyl (5.7 nmol) and one rat out of four that received lofenatanil (0.6 nmol) showed dysfunction. After epidural and intravenous injections of lofenatanil (0.6 nmol), the most common behavior was sliding from the inclined plane or nondirectional jumping to the floor.

Catalepsy was observed after 1 day of catheterization with epidural morphine (30 nmol, 90 nmol), fentanyl (5.7 nmol), and lofenatanil (0.6 nmol) and with intravenous fentanyl (5.7 nmol) and lofenatanil (0.6 nmol).

Truncal rigidity was observed after intravenous administration of fentanyl (5.7 nmol) and lofenatanil (0.6 nmol) and after epidural administration (1 day of catheterization) of lofenatanil (0.6 nmol). Tail rigidity was observed after epidural (1 day of catheterization) adminis-
tration of fentanyl (5.7 nmol) and lofentanil (0.6 nmol) and after intravenous administration of lofentanil (0.6 nmol).

After intravenous injection of lofentanil (0.6 nmol), three out of four rats displayed abdominal breathing and bradypnea (RR: 20–64 breaths/min) for about 1–5 h. The normal rat respiratory frequency is 66–114 breaths/min. After epidural injection (1 day of catheterization) of lofentanil (0.6 nmol), no rats displayed abdominal breathing.

DADL injected epidurally 1 day after catheterization produced no effect on motor function and none of the side effects mentioned above.

**PATHOLOGY**

Gross observation after 1 day of catheterization showed the beginning of a fibrotic reaction of brownish appearance around the catheter in 36 out of 43 rats. The tip of the catheter was obstructed in only two cases. In contrast, after 10 days of catheterization, a thick fibrotic reaction obstructing the catheter tip was observed in 31 out of 33 rats. In this last group, injection of methylene blue showed no spread in the epidural space (fig. 1E). In some of those rats, the skin along the catheter was opened in order to observe the catheter path during injection of methylene blue. The dye was seen to fill the lumen of the catheter and then the fibrotic sheath around the catheter, making a blue spot on the skin if the injection was continued. Depending on the volume of the fibrotic pocket around the cranioplastic cement, volumes varying from 80 to 300 μl were necessary to fill out both the catheter and the fibrotic sheath. Moreover, a mild deformation of the dura was observed in all animals.

Microscopic examination in three rats that received no drugs is shown in figure 5 after 1, 2, and 10 days of catheterization, respectively. After 1 day, the catheter was surrounded by several layers of red blood cells (figs. 5A and B). After 2 days, the red blood cells were still present although scattered by edema and proliferating connective fibers (figs. 5C and D). After 10 days, only connective tissue and a few lymphocytes were present around the catheter (figs. 5E and F). In these three rats, granulomas, giant cells, and fat were not observed in the epidural space.

**Discussion**

The present study demonstrates the characteristics of a model that permits reliable epidural injections in the rat. The characteristics are as follows: the time course, the pharmacology of the effects, and the development of fibrosis around the catheter. Estimations of ED₅₀ values, slopes, and antagonism of drug effects in rats with catheters implanted for 1 day are valid because the fibrotic reaction around the catheter is minimal and the tip does not appear obstructed. In contrast, after 10 days, an ef-

| Table 3. ED₅₀ (nmol), Slope Values, 90% Confidence Intervals, and Correlation Coefficients (r) for Epidurally Administered Bupivacaine, Morphine, Fentanyl, Lofentanil, and DADL on the Hot Plate (HP) and Tail Flick (TF) Tests in the Rat after One Day of Catheterization |
|---------------------------------|---|---|---|---|---|---|---|
|                                | HP  | TF  | HP  | TF  | HP  | TF  | HP  | TF  |
| Bupivacaine Hydrochloride       | 106.6 | 524.8 | 4.9  | 6.6  | 1.09 | 0.37 | 0.063 | 0.07  |
| Morphine Sulfate                | 40.9  | 292.4 | 3.1  | 4.9  | 0.64 | 0.15 | 0.043 | 0.02  |
| Fentanyl Citrate                | 278   | 941.9 | 7.7  | 8.7  | 1.8  | 0.88 | 0.093 | 0.25  |
| Lofentanil Oxalate              | 42.0  | 72.2  | 70.3 | 81.3 | 51.3 | 41.3 | 100.6 | 38.3  |
| DADL                            | ±50.8 | ±55.0 | ±34.9 | ±24.9 | ±20.6 | ±27.3 | ±65.0 | ±55.5  |
| 90% CI of the Slope             | ±50.8 | ±55.0 | ±34.9 | ±24.9 | ±20.6 | ±27.3 | ±65.0 | ±55.5  |
| r                               | 0.38  | 0.56  | 0.76 | 0.78 | 0.76 | 0.58 | 0.81  | 0.40  |
|                                 | 0.62  | 0.73  |
effective barrier to drug distribution develops. The histologic evidence presented here confirms the pharmacologic results.

**Time Course of Epidural Drug Action**

The time course of the onset of drug action likely reflects the time required for drug to move from the site of injection across the neural membranes to reach the site of action, while duration of action depends upon clearance and metabolism as well as binding properties of the ligand at the opiate receptor.

Epidurally administered drugs potentially enter the neuraxis by three different pathways: 1) directly across the dura into the cerebrospinal fluid (CSF); 2) through the perineurium of the mixed spinal nerves into the subperineural space, and then centripetally along the nerve roots to the spinal cord and subarachnoid space; or 3) by rapid uptake into the posterior radicular branch of spinal segmental arteries or into the epidural veins. Crock and Yoshizawa showed that the arteries, although small, reach the spinal cord in a potentially segmental fashion. Epidural venous absorption would increase blood levels of the drug and result in spinal and supraspinal action.

**Time of Onset.** In the present study, all drugs with high lipophilicity display a relatively rapid onset (fentanyl = lofentanil = bupivacaine > morphone = DADL). Molecular diffusion through extracellular channels, such as represented by the dura, varies inversely with the square root of molecular weight and is a function of the effective shape of the molecule but is not influenced by the lipid partition coefficient. As shown in table 4, the differences in molecular weight are minor and, therefore, cannot explain the very different effects seen with the five drugs.

To the degree that dural transfer is important, the delayed onset of action of DADL after epidural injection may be due to its linear peptide structure, which would result in bulky hinderance to the diffusion of this agent. In contrast, the relative similarity of the molecular configuration of the opiate drugs and their comparable dural permeability indicates that for these agents other factors are also important. The rapid onset of lofentanil and fentanyl as compared with morphine may be due either to their rapid absorption into the vasculature or to their ability to diffuse in neuronal tissue (after having crossed the dura). Movement in neural tissue or into blood vessels has been shown to be closely correlated with the lipid partition coefficient of the molecule. As will be noted below, those opioid agents with rapid onset also show a considerable effect when given intravenously.

**Duration of Action.** As shown in figure 2 and table 2, the rank order of duration of action after epidural injection on TF is as follows: lofentanil = morphine > fentanyl = bupivacaine > DADL. With regard to the structure-activity relationship of the opiate drugs, comparable data have been observed after IT injection of several of those agents in rats and primates. The long duration of action of morphine is likely due to its hydrophilicity and relatively delayed clearance from the CSF space. Regarding lofentanil, the binding complex between this agent and the stereospecific receptor is very stable, as evidenced by the extremely slow dissociation rate in vitro. In addition, the significant nonspecific binding observed during ligand competition studies suggests significant sequestration into the lipid phase. Both properties can account for the long

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† Yaksh TL, unpublished observations.
Fig. 5. Histologic sections of vertebral columns of three epidurally implanted rats that did not receive any drug. After 1 day of catheterization, the epidural catheter is essentially surrounded by red blood cells (A and B, L-3 section), after 2 days by edema (C and D, L-4 section) and after 10 days by connective tissue (E and F, L-4 section). The areas in the black squares on A, C, and E (original magnification, ×50) are shown on B, D, and F (original magnification, ×300). Granulomas, giant cells, and fat are not present.
<table>
<thead>
<tr>
<th></th>
<th>Morphine Sulfate</th>
<th>Fentanyl Citrate</th>
<th>Lofentanil Oxalate</th>
<th>DADL</th>
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<tbody>
<tr>
<td></td>
<td>HP</td>
<td>TF</td>
<td>HP</td>
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<tr>
<td><strong>ED₅₀ (nmol)</strong></td>
<td></td>
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<tr>
<td>EPI 1</td>
<td>4.9</td>
<td>6.6</td>
<td>1.09</td>
<td>0.37</td>
</tr>
<tr>
<td>IT†</td>
<td>12.6</td>
<td>17.7</td>
<td>4.6</td>
<td>3.6</td>
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<tr>
<td><strong>MW</strong></td>
<td></td>
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<tr>
<td>pKₐ octanol</td>
<td>669</td>
<td>528</td>
<td>7.9</td>
<td>8.4</td>
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<tr>
<td>water partition coefficient‡</td>
<td>1.42</td>
<td>813</td>
<td>1.450</td>
<td>—</td>
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</tbody>
</table>

— not available.

* Yaksh *et al*.,¹⁵ (For morphine, fentanyl and lofentanil, ED₅₀ confidence intervals (90%) overlap: for DADL, they do not.)

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<tbody>
<tr>
<td><strong>Duration</strong></td>
<td><strong>Action</strong></td>
<td></td>
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<td><strong>of action</strong></td>
<td><strong>in vivo</strong></td>
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| Fentanyl       | Displayed a significantly shorter duration of action than either morphine or lofentanil. The fact that at pH 7.4, less than 10% of fentanyl is un-ionized partly may explain these differences. The relatively low potency and duration of action of DADL differ from reports of its effect after IT injection and likely reflect a more limited access to the receptor site after epidural injection. Comparable results have been observed with other peptides after epidural injection in primates.⁶

**Pharmacology of Epidural Agents**

For the opiate drugs, the relative activity of the agents observed in the present work after epidural administration is comparable to that reported after systemic⁵ or IT administration.⁶ With regard to DADL, the ED₅₀ of this agent after epidural administration shows a 50-fold increase over its effects observed after IT injection.⁵ As discussed above, we believe this reflects a difference in disposition of the peptide versus the opiate drugs.

At epidural doses producing a maximal response on HP and TF, absence of normal withdrawal of forepaws during pinching was noted after fentanyl (four out of five) and lofentanil (four out of four) but not after bupivacaine (zero out of five) and morphine (zero out of four) administration (table 1). Regarding rostral spread, however, no conclusion can be made because of the possible vascular redistribution that may occur with these last two agents after epidural administration. This issue is discussed below.

As shown in figure 4, antagonism by naloxone of epidurally administered opiate drugs suggests that the analgesia is due to specific opiate–receptor interaction. However, naloxone (1 mg/kg ip) administered 5 min before epidural injection of lofentanil (0.6 nmol) did not antagonize the analgesia on TF, whereas naloxone (10 mg/kg ip) did. This may be explained by the extremely potent agonistic activity and strong binding to the opiate receptor of lofentanil.⁴ Comparable resistance to naloxone antagonism also is observed after intrathecal administration of this agent in the rat⁶ and in the primate.§ This observation suggests that great caution is required regarding the possible clinical use of epidural lofentanil.

**Epidural versus Systemic Routes**

Like Bromage’s study on human volunteers, the analgesic and side effects of morphine are negligible by the iv route when compared with the epidural route in the rat.¹⁷ In contrast to morphine, analgesia and side effects are comparable in the iv and EPI 1 groups for both fentanyl and lofentanil (tables 1 and 2). These two drugs are highly lipophilic. The increase in lipophilicity, if not important for dural permeability, likely leads to a more rapid transfer into epidural vessels. In human beings, Wolfe and Davies showed significant fentanyl plasma levels after epidural injection, although not large enough to explain the actions of fentanyl as being due to diffusion into epidural veins.¹⁸ However, epidural fat was not present in this strain of rats. Therefore, transfer of drug into fat cannot occur, and the amount of drug available for vascular absorption may be increased significantly. Epidural vascular absorption thus may explain the comparable analgesia and side effects in the iv and EPI 1 groups for fentanyl and lofentanil in rats. Furthermore, in comparison with all other groups, central depression after iv lofentanil seems most severe with abdominal breathing, bradypnea, and significantly more intense analgesia observed on TF. Lofentanil probably crosses the blood–brain barrier more efficiently than the other narcotics because of its very high lipophilicity.

**Catheter Enclosure**

Fibrosis around an epidural catheter was previously described in dogs by Lebeaux.¹⁹ Bromage considers the phenomenon as a possible cause of drug tolerance in hu-

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§ Yaksh TL: Unpublished observations.
man beings after repeated epidural injections. However, the fibrotic process has not been investigated systematically. Duce et al. demonstrated that the responses to epidural lidocaine in cats were not statistically different over periods of several weeks. However, the animals were tested for the first time 5–10 days after implantation. In the present experiment, animals with catheters implanted for 10 days had a complete fibrotic sheath around catheters and tips, both grossly and microscopically (figs. 1E, 5E, and 5F). Methylene blue injected epidurally at that time did not spread in the epidural space but returned in the fibrotic sheath. We believe that the fibrotic process plays a major role in the significant reduction of drug response and side effects over time (tables 1 and 2). Fibrin sleeve formation also has been described around intrathecal and intravenous catheters and around those subcutaneous silicone rubber implants. The role played by fibrosis in the development of tolerance to narcotics during chronic catheterization should be investigated further. Furthermore, acute tolerance or tachyphylaxis (e.g., after repeated epidural injection of local anesthetics) could be explained by a change in the diffusion properties of the dura and/or by a local destruction of the drug due to the foreign body reaction. In addition, the same experiments should be repeated with Teflon®, nylon, and silicone catheters to see if there is a substance time dependency on the degree of tissue reaction.

In conclusion, the rat model likely mimics a phenomenon occurring in humans and may be useful for studying the fibrotic process and the acute administration of epidurally administered drugs. These data, however, suggest that the epidural rat preparation may not be used for chronic studies of epidurally injected drugs.

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References