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Long Term Catheterization of the Lumbar Epidural Space in Rats

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VAN DEN HOOGEN, R. H. W. M. AND F. C. COLPAERT. *Long term catheterization of the lumbar epidural space in rats.* PHARMAC. BIOCHEM. BEHAV. 15(3) 515–516, 1981.—This note describes a simple technique for the administration of drug solutions into the lumbar epidural space of the unanesthetized and freely moving rat. A poly-ethylene (PE-10) catheter is inserted into the epidural space through a hole in the third lumbar vertebra, and subcutaneously tunneled towards the neck region. The implantation procedure is well-tolerated, and repeated saline injections over a prolonged period of time were found not to exert any deleterious effect on the animals' apparent health or gross behavior. The viability of the technique is demonstrated in an experiment on the effect of injection volume on the analgesic action of epidurally administered fentanyl.

Epidural injection Rat epidural space Fentanyl Analgesia

IN order to initiate pharmacodynamic studies of epidurally administered drugs in laboratory animals we developed a technique for long term catheterization of the lumbar epidural space in rats.

This technique offers some advantages over other animal techniques used to administer drugs to the spinal cord such as spinal catheters [8] or direct puncture methods [4]. There is less chance of damaging the spinal cord because of the strong ascensus medullae. The sacral part of the spinal cord extends to the cranial end of the third lumbar vertebra and from there on the coccygeal part runs to the cranial third of the fourth lumbar vertebra [2]. An intact dura mater protects the central nervous system against infections. Drug administration in the lumbar epidural space of rats has a closer relationship to the clinical practice of administering opiates epidurally in man for the purpose of treating pain of various aetiologies [1].

METHOD

Male Wistar rats weighing 250–300 g were anaesthetized by a subcutaneous injection of 1.5 ml Thalamonal®, followed by an intraperitoneal injection of 0.1% pentobarbital (3.5 mg/kg). After depilating the skin of the back the head of the animal was mounted in a stereotaxic instrument. The skin was sterilized with chloorhexidine and standard aseptic precautions were observed during surgery. A midline skin incision was made over the spinous process of the first five lumbar vertebrae. The identification of the lumbar vertebrae was made by palpating the tuber sacrales of the os ileum; the sixth lumbar vertebra lies between those two [2]. The fascia covering the superficial muscles of the back was opened. The long superficial muscles were carefully dissected from the lumbar vertebrae and retracted laterally causing as little trauma as possible. The m. interspinalis between the spinous processes of L3-L4 and L4-L5 were cut and removed. A 0.5 mm hole was drilled in the L4 spinous process and a 3.0 Tevdek ligature was passed through the hole. Using an electric dental burr, part of the spinous process and arch of L3 was removed, thus leaving a longitudinal groove in the vertebra. Finally, a small hole was made in the cranial end of the groove, which gave entrance to the lumbar epidural space. A polyethylene catheter (PE-10; nominal i.d. 0.28 mm) with a length of 20 cm was used. Before sterilization in 70% ethanol, the volume of the catheter was determined ("dead space") and a mark was placed 1 cm from one tip of the catheter. After sterilization, the catheter was dried and flushed with sterile saline. The curve of the spine was maximized by lifting the body with a forceps attached to the spinous process of L3 and the catheter was then gently introduced to a length of 1 cm cephalad into the lumbar epidural space. The catheter tip was now located at the level of L1. A loosely tightened half hitch was made in the catheter around the spinous process of L4. The Tevdek ligature was pulled through the half hitch and the catheter was attached to the spinous process with a reef knot. The area around the spinous process was covered with dental acrylic. The remainder of the catheter was subcutaneously tunneled

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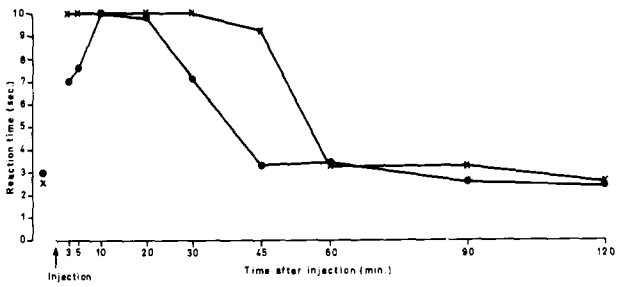

FIG. 1. Effects of 8 μg fentanyl administered epidurally on the tail-flick latency in the rat. Fentanyl was administered in a volume of either 1 (1×8 μg/μl; X–X) or 16 μl (16×0.5 μg/μl; ●–●). Each data point represents the median reaction time in seconds of 9 animals. The difference in the analgesic effect was statistically significant ($p<0.01$).

cephalad, the tip appearing in the neck region; here, a second half hitch was made in the catheter and covered with dental acrylic in order to prevent subcutaneous retraction. The skin incision was sutured in one layer, and the animal was given a subcutaneous injection of 0.5 mg/kg naloxone. At the end of the procedure the catheter was flushed with an amount of sterile saline equivalent to the volume of the dead space, and the catheter was blocked with a metal stopper. In skilled hands the whole procedure takes about 35 minutes. The animals were given at least one week to recover. During this time they were kept in individual cages with free access to food and water.

The following procedure was used to inject drug solutions and the amount of saline needed to flush the catheter. A manually operated gear driven syringe (Hamilton 700 series) with a long extension tube made of the same PE-10 material was filled with sterile saline. Then a small airbubble was aspirated into the extension tube followed by a volume of saline equal to the dead space of the rat's catheter. Then another small airbubble and finally the volume of the drug solution being studied was aspirated into the extension tube. After connecting this tube to the rat's catheter, the injection proceeded stepwise at a rate of 1 μl every 2 seconds. At least one hour prior to the injection the rat's catheter was flushed with 10 μl of air in order to empty it.

To demonstrate the viability of administering drugs epidurally by means of the technique described here an experiment was carried out on the effect of injection volume on the analgesic effect of 8 μg fentanyl. A group of 9 rats was used in two conditions. In one condition, 8 μg of fentanyl citrate was administered in a volume of 1 μl (concentration: 8 μg/μl); the injection volume in the second condition was 16 μl (concentration: 0.5 μg/μl). The sequence in which the two conditions were run, was randomized; two days elapsed in between the two injections. The tail flick procedure used here has been described in detail elsewhere [3]. Briefly, the rat was placed in a modified Bollmann cage with its tail hanging freely outside the cage. All readings were taken by a single observer and consisted of immersing the distal 5 cm of the tail into a warm (55°±1°C) water bath and determining the reaction time for its withdrawal to the nearest 0.1 sec. Readings were taken once before and 3, 5, 10, 20, 30, 45, 60, 90 and 120 min after injection. To avoid tissue damage, the cut-off time was limited to 10.0 sec.

RESULTS

While predrug latencies were almost identical in all animals, the postdrug latencies in the 8 μg/μl injection condition exceeded those in the 0.5 μg/μl group (Fig. 1). To further analyze this difference, analgesic effects were computed according to the method introduced by Winter and Flataker [7]. This was done by expressing area A as a percentage of area B [5]; area A represents the area, in minute-seconds, comprised between the time-effect polygon on the one hand and the predrug base line on the other; area B is comprised between the predrug base line and the zero level. The median % analgesic effect in the 8 μg/μl injection condition was 129.6 (95% confidence limits: 90.6–149.9) whereas that in the 0.5 μg/μl condition amounted to only 73.8% (43.0–86.5); this difference between the two injection conditions proved to be statistically significant ($p<0.01$, Wilcoxon test, two tailed) [6]. In another experiment (data not shown) 0.63 mg/kg of subcutaneous naloxone was found to prevent the analgesic effect of 8 μg of fentanyl given epidurally.

DISCUSSION

The technique described here provides a means of administering drugs into the lumbar epidural space of the rat via a chronically implanted catheter. After implantation of over 40 catheters and repeated saline injections no animal tested so far has shown any sign of apparent neurological damage. The initial experiment presented in this paper analyzes the effect of the same dose of fentanyl administered in two different volumes, on the tail-flick latency in the rat. The analgesic effect appeared to depend on the concentration of the drug at the level of the spinal segments which were to be blocked. This experiment thus demonstrates the viability of this technique for routine use in the laboratory rat.

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