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Epidural and Intravenous Sufentanil in the Rat: Analgesia, Opiate Receptor Binding, and Drug Concentrations in Plasma and Brain

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Doses of sufentanil (*i.e.*, 0.01, 0.04, 0.16, 0.63, 2.5, 10, and 40 μg/rat) were injected either into the lumbar epidural space or intravenously in rats weighing ± 250 g, and *in vivo* pharmacologic activities (*i.e.*, prolongation of latency to tail withdrawal in response to noxious heat, blockade of cornea and pinna reflexes, increase of skeletal muscle tone), *ex vivo* μ-opiate receptor binding (*i.e.*, displacement of specific ^{3}H-sufentanil binding in thalamus, striatum, hippocampus, cortex, mamillary body–medulla oblongata segment, medulla oblongata, and in cervical, thoracic, and lumbar spinal cord), and drug concentrations in plasma, brain, cortex, and cerebellum, were determined. An ED_{50} dose of intravenous sufentanil of 0.075 μg/rat produced analgesia. CNS-mediated *in vivo* side effects (*i.e.*, blockade of pinna and cornea reflexes, muscle rigidity) were apparent at 6–28 times higher doses. Epidural sufentanil also produced analgesia at an ED_{50} dose of 0.08 μg/rat, but CNS-mediated side effects occurred only at 35 to 76 times higher doses. This greater *in vivo* selectivity of epidural sufentanil in producing analgesia was consistent with *ex vivo* binding data that showed that in most areas of brain, but not in spinal cord, more μ-opiate binding occurs with intravenous than with epidural sufentanil. The two routes nonetheless differed by no more than a factor of approximately two in producing detectable levels of sufentanil both in plasma and in brain tissue. Analgesia produced by epidural sufentanil in rats may originate at least in part at μ-opiate receptor sites in the spinal cord; but the minute amounts of sufentanil that may reach the brain after epidural injection of low doses of the drug may perhaps amplify the spinal action. (Key words: Analgesics, Narcotics: sufentanil. Anesthetic techniques, epidural: opiates.)

IT HAS LONG BEEN RECOGNIZED[1-4] that spinal in addition to supraspinal mechanisms may be involved in opiate analgesia. More recently, opiate binding sites were shown[5-7] to exist in the spinal cord, and it was found[8,9] that analgesia can be produced in laboratory animals by intrathecal injection of opiates. These considerations have led to the intrathecal or epidural administration of opiates for the treatment of pain in humans.[10,11] The purpose of administering opiates *via* these routes has been to relieve pain in the relative absence of the additional effects of systemically administered opiates.

The ability of epidurally administered opiates to produce analgesia in humans has now been extensively documented.[12-14] It has also become apparent, however, that the analgesia that is so produced may be accompanied by a number of side effects. These may consist of pruritus, nausea, vomiting, urinary retention, and sedation,[14,15] but may also include respiratory depression.[16-18] These side effects presumably result from the transport of the epidurally administered opiate to sites in the CNS other than the target spinal cord segment.[19]

The fate of drugs that are administered epidurally is complex.[18,20,21] The drug may enter the cerebrospinal fluid (CSF) by direct penetration through the dura and also by diffusion *via* the perineural cuffs of the dura. The drug may also be removed from the epidural space by its uptake into epidural fat and by its absorption into the numerous extradural veins. Once the drug enters the CSF, it may diffuse along the spinal axis and be taken up by the nervous tissue as well as absorbed into the neurovasculature.[20-22] Opiates differ in their lipid solubility, binding affinity, and other characteristics that are likely to determine their fate after epidural injection. It may not be surprising, therefore, that differences occur among opiates in terms of their relative analgesic potencies and their side effects after epidural administration.[23,24]

Because of these and other considerations,[25] a need has become apparent for the detailed study of the mechanisms of the analgesia and the other effects of epidurally administered opiates.[19] We have developed a technique[26] for the catheterization of the epidural space in rat. Perhaps more than in other species, the availability of an epidural drug-administration technique in the rat permits basic studies to be carried out on the fate, effects, and mechanisms of action of epidurally administered opiates.

The present report presents one of several studies in which we have examined the *in vivo* actions (*e.g.*, analgesia, respiratory depression), mechanisms of action, and biologic fate of epidurally injected opiates in rats. Sufentanil is a newly developed, lipid-soluble, typical morphine-like compound[27,28] that selectively binds to μ-opiate receptors.[7,29] The experiments described here examined analgesia, opiate receptor binding, and drug concentrations in plasma and brain after epidural injection of various doses of sufentanil. The results obtained after epidural injection are compared with similar measurements made after intravenous injection.

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Materials and Methods

Animals

The animals used were 126 male Wistar strain rats weighing 250 ± 30 g on arrival from the breeding quarters. The animals were used only once, *i.e.*, with only one route and one pharmacologic treatment.

Experimental Design

Two series of experiments were conducted. In each case measurements were carried out after injection of saline (n = 14) or one of different doses (*i.e.*, 0.01, 0.04, 0.16, 0.63, 2.5, 10, and 40 μg/rat; n = 7 per dose) of sufentanil. The drug was given as an aqueous solution of the citrate; doses are expressed as the base.

In a first series, saline or sufentanil was given as a 0.2-ml bolus injection into the tail vein. In the second series, saline or sufentanil was injected stepwise into the lumbar epidural space in a volume of 20 μl as subsequently described.

All *in vivo* measurements were taken once before and 5 and 15 min after the intravenous or epidural injection. Immediately thereafter, the animals were killed by decapitation and the mixed venous–arterial blood that flowed out of the trunk was collected; brain areas and spinal cord were rapidly dissected, cooled in ice-cold buffer, and immediately further processed for the subsequently specified studies.

Epidural Catheterization

The animals were anesthetized with a subcutaneous 1.5 ml injection of Thalamonal® (Innovar®; 2.5 mg droperidol and 0.05 mg fentanyl per ml) followed by an intraperitoneal injection of sodium pentobarbital, 3.5 mg/kg. The animal was mounted in a David Kopf stereotaxic instrument after the skin of the back had been depilated. The skin was sterilized with iodine and standard aseptic precautions were observed during surgery. A midline skin incision was made over the spinous process of L-3, L-4, L-5, and L-6. The identification of the lumbar vertebrae was made by palpation of the iliac crest of the os ileum; the sixth lumbar vertebra lies between those two points. 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 with as little trauma as possible. The interspinalis muscle between the spinous processes of L4–L5 and L5–L6 was cut and removed. A 0.5-mm hole was drilled in the L-5 spinous process and a 3.0-mm Tevdek® ligature was passed through the hole. Using an electrical dental burr, the spinous process and part of the arch of L-4 were removed, thus leaving a longitudinal groove in the vertebra. Finally, a small hole was made in the cranial end of the groove that gave entrance to the lumbar epidural space after the yellow ligament had been perforated. A polyethylene catheter (PE-10; nominal 0.28 mm ID) with a length of 20 cm was used. Before sterilization in 70% ethanol, the volume of the catheter ("dead space") was determined 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 increased by lifting the body with a forceps attached to the spinous process of L-3 and the catheter was then gently introduced to a length of between 0.5 and 1 cm cephalad into the lumbar epidural space. The catheter tip was now located at the level of L-3. A gently tightened, half-hitch knot was made in the catheter around the spinous process of L-5. The Tevdek® ligature was pulled through the half-hitch knot 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 cephalad, the tip appearing in the neck region; here, a second half-hitch knot 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 1.25 mg/kg injection of naloxone HCl. The naloxone injection served to antagonize the respiratory depression that could possibly be produced by the fentanyl that was contained in the Thalamonal®. 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 plugged with a metal stopper. The animals were given 1 week to recover. During this time they were housed individually in standard rodent cages and had 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 air bubble was aspirated into the extension tube followed by a volume of saline equal to the dead space of the rat's catheter. Then a second small air bubble was aspirated and, finally, the volume of the drug solution being studied was aspirated into the extension tube. After connecting this tube to the catheter, the injection proceeded stepwise at a rate of 1 μl every 2 s; the injection volume was 20 μl. At least 1 h prior to the injection the catheter was flushed with 10 μl of air in order to empty it.

Analgesia Assay

A modification[30] of the tail-flick procedure was used to assay analgesic effects. Briefly, the rat was placed in a modified Bollman cage with its tail hanging freely outside

the cage. Readings consisted of immersing the distal 5 cm of the tail into a warm (55 ± 1° C) water bath and determining the reaction time for tail withdrawal to the nearest 0.1 s. Readings were taken one time prior to and 5 and 15 min after injection. The choice of these time intervals was based on preliminary experiments that had indicated that the effects of epidural sufentanil on the rat tail flick are maximal during this period of time after injection. These time intervals also covered the time of analgesic peak effect of intravenous sufentanil in rats.[27] Cut-off time was 10.0 s.

Other *In Vivo* Actions

Five and fifteen minutes after injection, observations were made of muscle tone and of cornea and pinna reflexes. Overall skeletal muscle tone was scored 0 (normal tone) to 3 (lead pipe rigidity); scores 1 and 2 represent weakly and moderately increased tone, respectively. The pinna reflex consisted of a characteristic head twitch and was scored 0 (normal reflex) to 3 (absence of any apparent motor response), depending on the response of the animal to gentle mechanical stimulation by means of a flexible metal rod (0: 0.5 mm); scores 1 and 2 indicate the reflex to be slightly or markedly retarded. The cornea reflex was examined in a similar manner, and was also scored 0 (normal reflex) to 3 (absence of any apparent motor response).

Radioligand Binding Assay

Occupation of μ-opiate receptor sites[7] was measured using ^{3}H-sufentanil in an *ex vivo* receptor-binding technique. Rats were decapitated 20 min after drug treatment and brain areas and spinal cord were immediately dissected.[7] The mamillary body–medulla oblongata segment was taken as a 2-mm thick, vertical slice of the medulla oblongata at the level where the mamillary bodies were localized by inspection under a dissection magnifying glass. This part of the medulla oblongata was assayed separately, because it was previously found to contain a high opiate-receptor density.[7]

Tissues were immediately cooled in ice-cold TRIS-HCl buffer (50 mM, *p*H 7.4) and within 10 min after decapitation homogenized with an Ultra Turrax® in a dilution of 40 v/w (w = wet weight of tissue). Membranes were collected by centrifugation at 25,000 *g* × 10 min in a refrigerated Sorvall® centrifuge.

The membrane fraction was resuspended in TRIS-HCl buffer (50 mM, *p*H 7.4) in a dilution of 200 v/w for brain areas and 100 v/w for spinal cord and samples were immediately taken for incubation. Assays were usually completed within 60 min after the death of the animal.

Incubation mixtures were composed of 2 ml membrane suspension; 0.1 ml solvent or dextromoramide in 10% ethanol (final concentration in the incubation mixture: 500 nM); and 0.1 ml ^{3}H-sufentanil in 10% ethanol (final concentration in the incubation mixture: 0.5 nM). Incubation was run for 15 min at 37° C. Labeled membranes were harvested and rinsed with 2 × 5 ml ice-cold buffer by rapid filtration under suction, using Whatman GF/B® glass fiber filters and a 40-well multividor (Janssen Scientific Instruments Division, Beerse, Belgium). Radioactivity retained on the filter was counted in a liquid scintillation spectrometer.

Specific binding was given by the difference in radioactivity counted on the filters in assays in the absence and the presence of dextromoramide, the active member of a pair of enantiomers.[7]

In each tissue preparation, binding measurements were done in triplicate. ^{3}H-sufentanil (specific radioactivity: 15 Ci/mmol) was obtained from Janssen Life Sciences Products, Beerse, Belgium. Radioactive purity as determined by high-performance liquid chromatography (HPLC) was 99.6%.

Drug Concentration in Plasma and Brain

Concentrations of sufentanil in plasma and homogenates of the cerebral cortex and the cerebellum were determined with a specific and sensitive radioimmunoassay method that has been described and validated elsewhere.[31] The specificity of the assay has been demonstrated by a combined gas chromatography/mass spectrometric method.[32] Because tissue homogenates had to be extracted prior to radioimmunoassay, plasma samples were analyzed in a similar manner. The extraction procedure was as follows: duplicate aliquots of 0.5 ml plasma were made alkaline with 0.5 ml of 1 M sodium hydroxide and extracted twice with 2 ml of heptane-isoamyl alcohol (95: 5). The combined organic layers were extracted with 3 ml of 0.05 M sulphuric acid and discarded after centrifugation. The aqueous layer was made alkaline with 0.15 ml of concentrated ammonia and extracted with 5 ml of heptane-isoamyl alcohol. The organic layer was separated and evaporated to dryness under nitrogen. The residue was reconstituted with 0.05 ml of methanol and 0.55 ml of 2% bovine serum albumin (w:v) 0.05 M phosphate buffer (*p*H 7.5) and aliquots of 0.05 and 0.5 ml were subjected in duplicate to radioimmunoassay.[31] By using these assay conditions,[31–33] the detection limit of sufentanil in plasma was 0.1 ng/ml. Aqueous homogenates of cerebellum were prepared using an Ultra Turrax® in a dilution of 1:4 (w:v) and aliquots of 1 ml of the homogenates containing 200 mg of tissue were extracted according to the procedure used for plasma. Aliquots of 2 ml (containing 200 mg of tissue) were obtained from a first homogenate of the cortex (1:10, w:v), prepared for the radioligand binding assay, and extracted prior to radioimmunoassay as described for plasma. The detection limit was 0.5 ng/g of wet tissue.

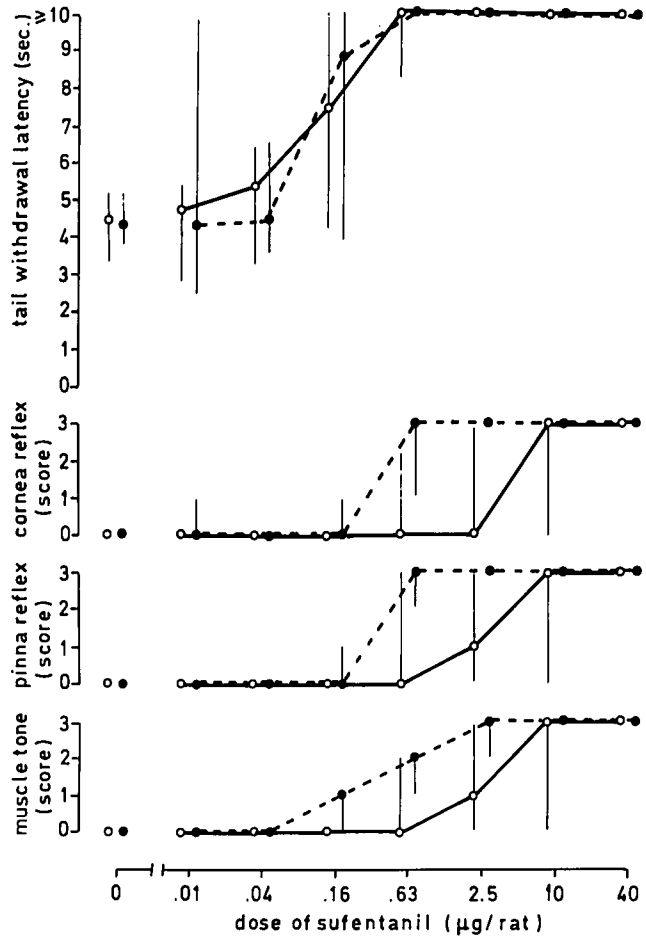

FIG. 1. Analgesia, blockade of cornea and pinna reflexes, and production of skeletal muscle rigidity after epidural (O —— O) or intravenous (● - - - ●) injection of sufentanil in rats. Analgesia was measured as the latency (to the nearest 0.1 s) to tail withdrawal from a 55° C warm water bath. Cornea and pinna reflexes and muscle tone were scored 0 to 3 as detailed in "Methods". Data points represent the median (and 95% confidence limits) of 14 saline control animals (dose 0) or of n = 7 for each dose of sufentanil. All data were analyzed in terms of the ED_{50} doses that were required to produce criterion values of effect (see "Methods"). Analysis reveals intravenous sufentanil to be more potent than epidural sufentanil in producing blockade of the pinna and cornea reflexes; differences were not significant ($P > 0.05$) with regard to analgesia and muscle tone (table 1).

STATISTICAL METHODS

The experiments examined a total of 16 different dependent variables. Criterion values were defined for every single variable, and data obtained after sufentanil administration were transformed into the percentage of rats (out of n = 7 per dose with both routes) that satisfied this criterion. This percentage was then used to compute ED_{50} values according to the method of Finney.[34] Differences between ED_{50} values were evaluated according to the method of Litchfield and Wilcoxon.[35]

The criterion values being used were as follows. Tail withdrawal latency was evaluated using the >6.0-s as well as the >10-s criterion. Score 3 was the criterion used to evaluate blockade of pinna and cornea reflexes as well as muscle tone. Binding of specific ^{3}H-sufentanil was considered to be inhibited if the amount of radioactivity in the experimental animal was lower than that of the lowest value obtained in the saline (control) group; note that the control level of radioactivity differed among the six areas of brain and the three sections of spinal cord being examined, and that criterion values differed accordingly. Criterion levels for drug concentration were 0.1 ng/ml in plasma and 0.5 ng/g in brain tissues; lower concentrations were not detectable.

Results

Results are presented (figs. 1–3) as the median and 95% confidence limits. With each of the 16 indices that were examined, data were analyzed in terms of the percentage of animals that satisfied the criterion that is specified in "Methods". This permitted ED_{50} values to be computed[34] (table 1), so that comparisons among indices and between routes can be made in terms of these ED_{50} values.

ANALGESIA AND OTHER *IN VIVO* ACTIONS

In none of the 26 animals used did the preinjection latency exceed 6.0 s (not shown). Postinjection *in vivo* measurements are reported (fig. 1) as the highest of the two latencies or scores that were obtained at 5 and 15 min after injection. Postinjection latency also failed to exceed 6.0 s in any of the 28 rats that received saline *via* either the epidural or the intravenous route. Postinjection scores for cornea and pinna reflexes and for muscle tone were 0 in all control animals.

On epidural injection, the 0.01-μg/rat dose of sufentanil exerted no apparent effect on the latency for tail withdrawal. Latency exceeded 6.0 s in two of seven rats that had received 0.04 μg/rat, and increased further orderly as a function of dose at higher doses (fig. 1). Using the >6.0-s latency as a criterion for analgesic drug activity,[30] the ED_{50} value of epidural sufentanil was 0.08 μg/rat. Using the ≧10-sec latency as a criterion, sufentanil's ED_{50} was 0.48 μg/rat, or six times the >6.0-sec dose (table 1). Epidural sufentanil also blocked (score 3) the pinna and cornea reflexes and induced rigidity (muscle tone score 3) in a dose-dependent manner; the ED_{50} values that were obtained for these effects exceeded the analgesic ED_{50} by a factor 35, 62, and 76, respectively.

The ED_{50} of intravenous sufentanil in producing analgesia (>6.0 s) was 0.075 μg/rat, while only a 2.5-fold higher dose was required to obtain latencies greater than 10 s. The doses at which pinna and cornea reflexes were

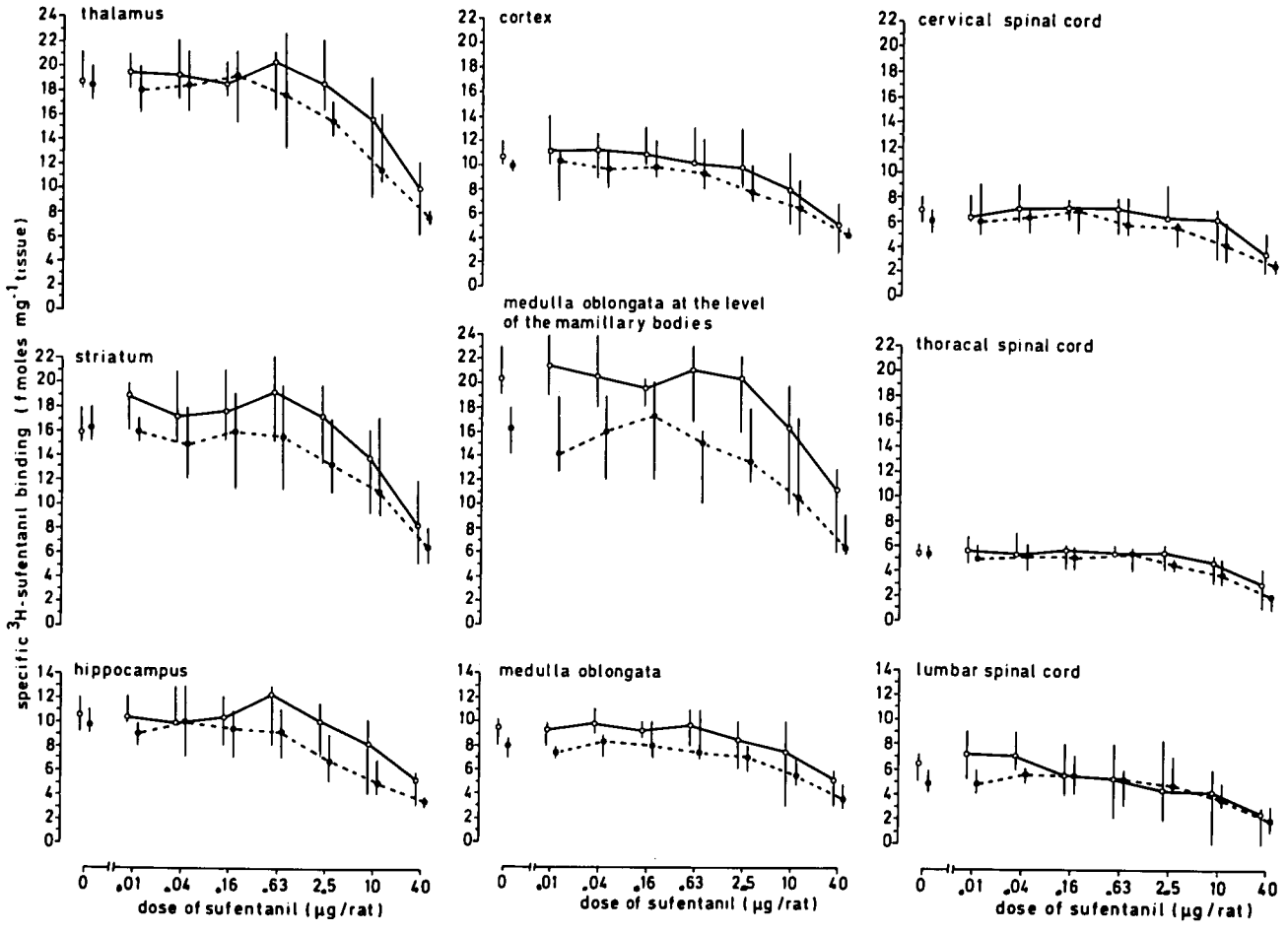

FIG. 2. Specific ^{3}H-sufentanil binding, in fmol/mg of wet tissue, measured *ex vivo* in six areas of brain and three sections of spinal cord of rats that received saline or one of several doses of sufentanil *via* either the epidural or intravenous route. See also figure 1 legend.

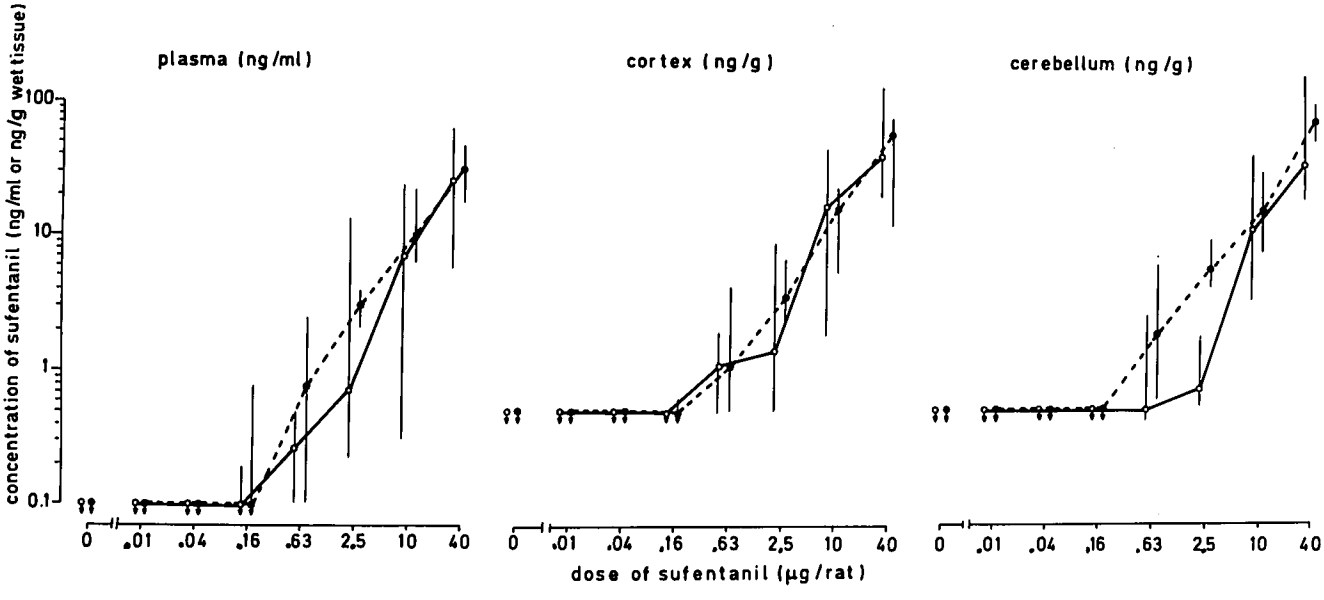

FIG. 3. Concentration of sufentanil in plasma, cortex, and cerebellum in rats 20 min after epidural or intravenous injection of sufentanil. The detection limits were 0.1 ng · ml^{-1} of plasma and 0.5 ng · mg^{-1} of wet brain tissue. See also figure 1 legend.

TABLE 1. *In Vivo* Pharmacologic Activity, Receptor Binding in Brain and Spinal Cord, and Concentration in Plasma and Brain after Epidural (ED) or Intravenous (IV) Injection of Sufentanil in Rats

Parameter	Epidural		Intravenous		$\frac{ED_{50}\text{-ED}}{ED_{50}\text{-IV}}$
	ED_{50}	Ratio	ED_{50}	Ratio	
Analgesia and Other *In Vivo* Actions					
Tail withdrawal > 6 s	0.08 (0.041–0.16)	1	0.075 (0.029–0.19)	1	1.1
Tail withdrawal ≥ 10 s	0.48 (0.02–1.2)	6	0.19 (0.050–0.72)	2.5	2.5
Pinna reflex	2.8 (0.93–8.7)	35	0.48* (0.064–3.6)	6	5.8
Cornea reflex	5.0 (2.9–8.5)	62	0.67* (0.35–1.3)	9	7.5
Muscle tone	6.1 (2.7–14)	76	2.1 (0.55–8.2)	28	2.9
***Ex Vivo* Inhibition of Specific ^{3}H-sufentanil Binding in Brain and Spinal Cord**					
Thalamus	2.6 (0.68–10.2)	32	1.0 (0.27–3.7)	13	2.6
Striatum	6.1 (2.9–13)	76	0.92* (0.17–4.9)	12	6.6
Hippocampus	8.7 (3.9–20)	109	0.77* (0.26–2.3)	10	11
Cortex	5.0 (2.5–9.9)	62	1.2 (0.28–4.9)	16	4.2
Mamillary bodies	8.8 (2.8–27)	110	3.1 (0.51–19)	41	2.8
Medulla oblongata	8.7 (3.8–20)	109	9.4 (5.3–17)	125	0.93
Cervical spinal cord	4.9 (0.55–43)	61	5.0 (2.5–9.9)	67	0.98
Thoracic spinal cord	6.7 (1.4–31)	84	7.5 (1.3–43)	100	0.89
Lumbar spinal cord	3.1 (0.66–14)	39	8.5 (3.1–24)	113	0.36
Concentration of Sufentanil in Plasma ($ng \cdot ml^{-1}$) and Brain ($ng \cdot g^{-1}$)					
Plasma	0.26 (0.12–0.55)	3.2	0.17 (0.070–0.43)	2.4	1.5
Cortex	0.61 (0.27–1.4)	8	0.32 (0.16–0.63)	4.3	1.9
Cerebellum	0.67 (0.35–1.3)	8	0.32 (0.16–0.63)	4.3	2.1

ED_{50} values (and 95% confidence limits; in μg/rat) of ED and IV sufentanil in producing analgesia and other *in vivo* actions, in inhibiting specific ^{3}H-sufentanil binding *ex vivo* in areas of brain and spinal cord, and in occurring at detectable concentrations in plasma (0.1 $ng \cdot ml^{-1}$) and brain (0.5 $ng \cdot g^{-1}$). Also given is the ratio of the ED to the IV ED_{50} value.

ED_{50} values were computed according to the method of Finney[55] and compared according to the method of Litchfield and Wilcoxon.[42]

* Difference between the ED and IV ED_{50} doses is $P < 0.05$.

blocked and rigidity was induced exceeded the analgesic dose by a factor of 6, 9, and 28, respectively.

INHIBITION OF SPECIFIC ^{3}H-SUFENTANIL BINDING

The total number of specific ^{3}H-sufentanil binding sites differed considerably among the nine areas of brain and spinal cord that were examined (fig. 2). Levels were high (*i.e.*, 16–20 fmol/mg) in striatum, thalamus, and the mamillary body–medulla oblongata segment and were lowest (*i.e.*, 4–7 fmol/mg) in spinal cord.

Epidural sufentanil inhibited ^{3}H-sufentanil binding in all areas. The inhibition occurred at ED_{50} doses of 2.6 and 3.1 μg/rat in thalamus and lumbar spinal cord, respectively, and at consistently higher doses (*i.e.*, 4.9 to 8.8 μg/rat) in other areas. The inhibition of binding thus occurred at a level of dose that was similar to the 2.8 to 6.1 μg/rat doses at which *in vivo* actions other than analgesia were observed.

Intravenous sufentanil inhibited ^{3}H-sufentanil binding at about 1 μg/rat in thalamus, striatum, hippocampus, and cortex; this was also roughly the dose at which intravenous sufentanil blocked the pinna and cornea reflexes and induced rigidity. Binding in medulla oblongata and in spinal cord occurred only at 3–9-fold higher doses.

Comparison of the various areas examined reveals (table 1) that the thalamus is a preferred target for sufentanil to inhibit ^{3}H-sufentanil binding after both epidural and intravenous injection. Note that inhibition of binding in thalamus occurred at a dose that exceeded the ED_{50} for deep analgesia (≧10 s) by the same ratio (*i.e.*, 5.4 and 5.3, respectively) after both epidural and intravenous injection.

The doses of epidural sufentanil that were required to inhibit ^{3}H-sufentanil binding were (2.6–6.6-fold) higher than the intravenous doses in all areas of brain except the medulla oblongata. In contrast, the epidural dose inhibiting binding in lumbar spinal cord was 2.7-fold lower than the intravenous dose. The latter difference grew smaller (*i.e.*, to 1.1 and 1.0) as the section of cord being considered (*i.e.*, thoracic and cervical cord, respectively) was more distant from the lumbar site of epidural injection. Much like in thoracic and cervical spinal cord, and unlike in other brain areas, the epidural dose inhibiting binding in medulla oblongata was similar to the intravenous dose.

Concentrations in Plasma and Brain

The limit for detecting sufentanil was 0.1 ng/ml in plasma, and 0.5 ng/g in brain tissues. Both epidural and intravenous doses of sufentanil yielded detectable amounts of the drug in plasma and brain (fig. 3). The relationship of dose to concentration was similar with the two routes. The doses of epidural sufentanil that yielded a concentration greater than the detection limit were slightly but not significantly higher than the intravenous doses (table 1).

Discussion

The present study examined the analgesic effects of sufentanil on epidural and intravenous injection in the rat. The analgesia produced by sufentanil was compared with several other indices of CNS action of the drug: 1) the cornea and pinna reflexes are controlled by the fifth and tenth cranial nerves. The blockade of these reflexes is a characteristic effect of opiate drugs[36] and requires the drug to penetrate these sites in the brain. The reflexes were monitored in this study in an effort to obtain an *in vivo* measure of drug activity in the brain. Skeletal muscle tone was also monitored as an *in vivo* measure of opiate drug activity in brain; opiate rigidity is likely mediated by opiate receptors in the striatum[37] and substantia nigra.[38] Other variables were 2) *ex vivo* binding to μ-opiate binding sites[7] in different areas of brain and spinal cord; and 3) concentration of sufentanil in cortex and cerebellum and, also, in plasma.

Epidural and intravenous sufentanil were essentially equipotent in producing analgesia (*i.e.*, tail withdrawal latency > 6.0 s; table 1). Relative to the analgesic doses, 6–28-fold higher intravenous doses blocked the cornea and pinna reflexes and induced muscular rigidity; in contrast, 35–76-fold higher epidural doses were required to produce the same effects. This greater *in vivo* selectivity of epidural as opposed to intravenous analgesia was paralleled by the *ex vivo* binding data that were obtained in various areas of brain. With the exception of the medulla oblongata, doses that were 10 to 41 times the intravenous analgesic dose inhibited specific ^{3}H-sufentanil binding, while 32 to 110 times the epidural analgesic dose was required for the same effect. Also, the absolute epidural doses at which reflex blockade and hypertonia occurred (*i.e.*, 2.8 to 6.1 μg/rat) corresponded remarkably well to the doses (*i.e.*, 2.6 to 8.8 μg/rat) at which binding occurred in brain (table 1). This data thus indicate that relative to equianalgesic intravenous doses, epidural sufentanil produces less *in vivo* pharmacologic activity that originates from cerebral sites and suggest that this is due to less binding at those sites. However, care must be taken in the interpretation of the *ex vivo* binding data. Sufentanil is known to dissociate rapidly (half-life of dissociation at 37° C: 2.4 min) from opiate receptor sites. In spite of cooling, dissociation of the drug during the tissue preparation is likely to occur. Hence, the degree of receptor occupation measured *ex vivo* is probably lower than the degree of receptor occupation that may in fact have occurred *in vivo*. Using a slowly dissociating drug, lofentanil, in *ex vivo* binding studies following intravenous administration, it was found that complete analgesia was obtained when less than 10% of brain μ-opiate receptor sites were occupied by the drug.[39]

The spinal cord is the likely site of action of epidural sufentanil to produce analgesia,[19] and some of the findings obtained here are consistent with this view. Specifically, the lumbar spinal cord was the only area of CNS in which epidural sufentanil bound at two-fold lower doses than intravenous sufentanil. The difference between the two routes diminished as the spinal area being considered was more distant from the lumbar location of the catheter tip; the ratio of the epidural to the intravenous ED_{50} for binding increased from 0.36 at the lumbar level to 0.89 and 0.98 at the thoracic and cervical levels, respectively (table 1). This gradient suggests that, on epidural injection, sufentanil at least in part penetrates the dura, diffuses into the CSF, and then occurs in CSF at a concentration that is lower as the site is more distant from the site of injection. It is this process that is perhaps responsible for the fact that the medulla oblongata was the only area of brain in which a same degree of receptor occupation was obtained with the two routes.

Other findings, however, would seem to cast doubt on the notion that the analgesic action of epidural sufentanil is mediated solely by the spinal cord. First, while the lumbar spinal cord was the site of CNS in which the epidural–intravenous ratio of ED_{50} values was most favorable, the absolute ED_{50} of epidural sufentanil for binding in spinal cord was still 39 times higher than its analgesic ED_{50}. This dose (*i.e.*, 3.1 μg/rat) was, in fact, not lower than the 2.6-μg/rat dose at which epidural sufentanil bound in thalamus. It can be argued, however, that the binding assay is simply less sensitive than the tail-flick assay; the difference in sensitivity would be at least 39-fold as estimated from the epidural data, or 67–113-fold as estimated from the intravenous data (table 1). Second, after epidural injection, sufentanil was detectable in plasma at doses that were only 3.2 times higher than the analgesic dose. The dose at which epidural sufentanil was detectable in plasma was also 12 times lower than that at which binding in lumbar spinal cord occurred.

It is thought[40] that both spinal and supraspinal mechanisms may contribute to the analgesic action of systemically administered opiates. Specifically, a supraspinal action of the opiates is capable of amplifying the analgesia produced at spinal sites, and this amplification becomes apparent even at very low doses.[41] The following hypo-

thetical mechanism can be entertained, therefore, to account for the present data. Low doses of epidural sufentanil act on the spinal cord to produce some degree of analgesia. A fraction of the drug reaches the brain through blood and, perhaps, CSF; the drug concentration that is so reached in the brain is minute and may be difficult to detect[39] by either radioimmunoassay or *ex vivo* displacement of ligand binding. This minute concentration may be sufficient, however, to amplify the initial spinal action, so that a more marked analgesia develops. Opiate effects that originate exclusively in the brain may require much higher local concentrations of the drug and will therefore become apparent only at considerably higher doses.

One notable feature of the binding data is regional variation. On intravenous injection, for example, the dose that was required for receptor occupation to occur in thalamus, striatum, hippocampus, and cortex was virtually the same (*i.e.*, ranged from 0.77 to 1.2 μg/rat); but receptor occupation in the mamillary body–medulla oblongata segment and in medulla oblongata required 3–9-fold higher doses. Also, the magnitude of the differences between receptor occupation by epidural and intravenous sufentanil varied from a ratio of approximately one in medulla oblongata to as high as 11 in the hippocampus. The mechanisms of these rather complex regional variations are not immediately apparent; they may perhaps involve differences among tissues in vascularity and in the capacity to retain drugs.

In conclusion, the present data indicate that the analgesia produced by epidural sufentanil in rat occurs in the presence of less CNS-mediated, *in vivo* side effects than is the case with intravenous sufentanil. This greater *in vivo* selectivity of epidural sufentanil in producing analgesia was consistent with *ex vivo* binding data that showed that in most areas of brain, but not in spinal cord, more μ-opiate binding occurred with intravenous than with epidural sufentanil. The two routes nonetheless differed by no more than a factor of about two in producing detectable levels of sufentanil in plasma and brain. Analgesia produced by epidural sufentanil in rats may originate at least in part at spinal μ-opiate sites; however, the minute amounts of the drug that may reach the brain on epidural injection of low doses of the drug may perhaps amplify the spinal action.

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