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Behavioral and Histopathologic Effects Following Intrathecal Administration of Butorphanol, Sufentanil, and Nalbuphine in Sheep

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A large number of opioids and nonopioids have been administered epidurally and intrathecally in the hope of providing segmental analgesia without serious adverse effects. However, neurotoxicity data are generally unavailable for many of these drugs. The present study evaluated the behavioral, motor, electroencephalographic, and histopathologic changes following intrathecal injection of large and small doses of butorphanol, sufentanil, and nalbuphine in sheep. Thirty-two sheep (20–32 kg) were anesthetized and catheters placed intrathecally after hemilaminectomy. The large doses of butorphanol, sufentanil and nalbuphine were 0.375 mg/kg (4.4–5.2 ml), 7.5 μg/kg (3.6–4.8 ml) and 0.75 mg/kg (1.5–2.4 ml), and the small doses were 0.075 mg/kg (0.9–1.1 ml), 1.5 μg/kg (0.7–0.9 ml) and 0.15 mg/kg (0.38–0.5 ml), respectively. The opioids were administered intrathecally every 6 h for 3 days and the above-mentioned parameters studied. Five sheep received intrathecal saline (1.1 or 5.2 ml) and served as controls. Histopathologic changes were evaluated by a neuropathologist blinded to the study protocol. Irrespective of dose, intrathecal injection of butorphanol was associated with severe behavioral responses such as agitation, rigidity, vocalization, and restlessness, as well as prolonged or irreversible hindlimb paralysis. Electroencephalography showed increased cortical activity or seizure activity. One sheep died because of severe respiratory depression that did not respond to naloxone. Spinal cord histologic changes consisted of suppurative meningitis and myelitis as well as neuronal changes such as spongiosis and chromatolysis. Large doses of intrathecal sufentanil were associated with similar though somewhat less severe responses. The behavioral and motor changes following the small dose of intrathecal sufentanil were of mild to moderate nature. Following intrathecal nalbuphine, the above-mentioned changes were similar to those seen in control animals. We conclude that butorphanol in doses of 0.075 and 0.375 mg/kg intrathecally and sufentanil 7.5 μg/kg intrathecally are neurotoxic in sheep. (Key words: Analgesics, opioids: butorphanol; sufentanil; nalbuphine. Anesthetic techniques: spinal. Complications: neurotoxicity.)

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THE ADMINISTRATION OF OPIOIDS epidurally and intrathecally for pain management is well established. The principal problem with the technique is the occurrence of late-onset respiratory depression. It is believed by many that the risk of this rare but potentially dangerous complication is highest with hydrophilic morphine, and consequently a large number of lipophilic opioids and nonopioids (more than 20 different substances) have been studied clinically in the hope of providing effective analgesia without respiratory depression.[1–4] However, with few exceptions, there is a general lack of animal neurotoxicity data for most of the opioids that have been and are being used in humans.[5–7] Furthermore, because neurotoxicity for spinal drugs appears to be route-dependent, drugs intended for epidural administration may have serious consequences if they are inadvertently deposited intrathecally.

In the present study, butorphanol, sufentanil, and nalbuphine were administered intrathecally in sheep in two doses. The drugs were given every 6 h for 3 days with the following aims:

1. To study the behavioral, motor and electroencephalographic (EEG) changes.
2. To evaluate if these changes are dose-dependent.
3. To study the histologic changes in the spinal cord.

Large intrathecal volumes were administered to determine what would happen if an epidural volume were to be inadvertently injected intrathecally.

Materials and Methods

The study protocol was approved by the Animal Welfare Committee of University of Texas Medical School, Houston. Thirty-two sheep weighing 20–32 kg were included in the study. The sheep were housed in individual

portable metabolic cages and had free access to food and water.

Following induction of anesthesia with thiopental and tracheal intubation, the lungs were ventilated (Harvard respirator) with 1–2% halothane in oxygen; no parenteral opioids were administered. Sterile technique was used during surgery. Catheters were inserted into the femoral artery and femoral vein. A 4-cm paravertebral incision was made and a hemilaminectomy performed at the level of the L3 or L4 vertebra. The dura was exposed and a Tuohy epidural needle was introduced into the spinal dura. An 18-G catheter (Portex) was introduced in the subarachnoid space and advanced 4–5 cm in the rostral direction. Correct placement of the catheter was confirmed by aspirating spinal fluid through it. The catheter was secured in place with a suture and cyanoacrylate (Super Glue). The intrathecal catheter was tunneled subcutaneously and externalized close to the exit site for femoral arterial and venous catheters. To prevent the catheters from being dislodged during movement, the subarachnoid, femoral arterial, and venous catheters were all rolled into a plastic pouch, which was secured by sutures to the abdominal wall near the forequarters.

After closing the hemilaminectomy wound, general anesthesia was discontinued and the sheep allowed to recover. After recovery, which took 2–3 h, the correct position of the intrathecal catheter was reconfirmed by aspiration of cerebrospinal fluid (CSF). The sheep were randomly divided into four groups and assigned to receive intrathecal injection of one of the following: butorphanol, sufentanil, nalbuphine, or saline.

The study was carried out in two phases. In phase 1 the doses of opioids studied were five times those generally administered epidurally for management of pain in humans (table 1). In phase 2 the opioid doses corresponded to the therapeutic epidural doses reported in the literature.[8–10] The reason for performing the study in two phases was that the second phase would be unnecessary if the large doses of opioids (phase 1) did not show any evidence of neurotoxicity. However, because of major behavioral, motor and respiratory changes and the death of two animals (*vide infra*), the second phase of the protocol was carried out, as approved by the Animal Welfare Committee. Table 1 describes the volumes and doses administered during phases 1 and 2. The table also shows the number of sheep studied in different groups. Analgesic effects of the injected opioids were not evaluated in this study.

Commercially available preservative-free butorphanol (Stadol®, Bristol Laboratories) 2 mg/ml and sufentanil (Sufenta®, Janssen Pharmaceuticals) 50 μg/ml were used. Nalbuphine solution (10 mg/ml) was freshly prepared by mixing saline with the powder (Nubain®, Du Pont). All three opioids contain citrate salt; butorphanol also contains tartrate salt. The drugs were administered according to the protocol in table 1. The volume of injected drugs was 0.38–1.1 ml in the small-dose group and 1.5–5.2 ml in the large-dose group, depending on the weight of the animals (table 1). The injection time was 60–90 s. The volumes of injected drugs were kept similar by appropriate dilution.

Pulse, respiratory rate, and behavioral and motor changes were monitored every 10 min for 1 h after each injection. The animals were observed over the next 3 days for abnormalities in behavior, posture, and movement. The changes were graded on a four-grade scale where 0 = no changes and +++ = the most severe changes (table 2). In animals showing severe behavioral or motor changes, saline (same volume as that of the opioid that produced changes) was injected through the catheter after the animal recovered. This was done to ensure that the behavioral changes were drug-related and not due to barotrauma from the large volume of injectate. The volume of saline injected was identical to the largest volume of injectate in each group (table 1).

For monitoring of EEG changes platinum needle electrodes were placed bilaterally in a frontoparietal montage with a frontoparietal zero ground. Compressed spectral array (CSA), a format for processing the EEG signal, was recorded using a two-channel Neurotrac (Interspec Inc.,

TABLE 1. Study Protocol

Drug	Number of Animals	Weight (kg) Mean (range)	Dose	Total Dose Mean (range)	Volume of Injectate (ml) Mean (range)
Phase 1 (large dose)					
Butorphanol	6	25.6 (23.5–27.5)	0.375 mg/kg	9.6 mg (8.8–10.3)	4.8 (4.4–5.2)
Sufentanil	6	27.5 (23.7–32.0)	7.5 μg/kg	206 μg (178.5–239.3)	4.1 (3.6–4.8)
Nalbuphine	6	28.0 (20–32)	0.75 mg/kg	21.0 mg (15.0–24.0)	2.1 (1.5–2.4)
Saline (control)	3	29.5 (28–31)	—	—	5.2
Phase 2 (small dose)					
Butorphanol	3	25.3 (22.7–28.0)	0.075 mg/kg	1.9 mg (1.7–2.1)	1.0 (0.9–1.1)
Sufentanil	3	27.2 (22.4–30.5)	1.5 μg/kg	40.8 μg (33.6–45.8)	0.8 (0.7–0.9)
Nalbuphine	3	28.7 (25.3–31.3)	0.15 mg/kg	4.3 mg (3.8–4.7)	0.43 (0.38–0.5)
Saline (control)	2	—	—	—	1.1

TABLE 2. Grading of Behavioral and Motor Changes

Grade	Behavioral, Motor, Respiratory, and Cardiovascular Changes
0	Animal standing or sitting (about 16 h/day), eating most of the time (3–6 kg grain and some hay daily), drinking (4–6 l/day), ruminating, nosing objects; normal respiratory rate (12–15 breaths per min) and heart rate (100–120 beats per min).
+	Tonic contractions of neck muscles and rigid extention of forelimbs during injection which disappear after injection. Occasional baaing (vocalization). About 15–20% increases in respiratory and heart rate. These changes last about 15–20 min. Motor weakness of hindlimbs lasting 5–10 min, attempts at standing unsuccessful. Early motor recovery (30–60 min). Normal behavior pattern by 30 min and motor function by 1 h.
++	Whole body rigid during and up to 90 s after injection. Animal appears restless and agitated, kicks frequently with forelegs. Distress baaing every few minutes. Increases of 40–50% in heart rate and respiratory rates. Gradual recovery over a period of about 1 h followed by moderate sedation lasting 2–3 h. Decreased interest in surroundings and in eating, drinking, and ruminating. Animal sitting and lying for long periods (4–5 h/day). Prolonged motor weakness of hindlegs (1.5–5 h). Attempts by animal to stand unsuccessful. Normal behavior and motor function by 4–5 h after injection.
+++	Generalized total body rigidity and convulsions during and up to 90 s after injection. This is followed by flaccid paralysis of hindlegs and sudden falling of the animal within 1–2 min of injection. Severe agitation, violent kicking of forelegs, frequent distress baaing (every 1–2 min) lasting up to 30 min. Respiratory rates greater than 80 beats per min and heart rates greater than 200 beats per min during this phase. This is followed by gradually increasing sedation. Animal totally uninterested in surroundings or in eating or drinking. Respiratory rates below baseline values during this phase. The excitation phase lasts about 30 min and the sedation phase about 2–3 h. Hindleg paralysis irreversible. The animal does not attempt to stand. Attempts by observer (every 6 h) to help the animal stand are unsuccessful. Cutaneous noxious stimuli of hindlegs does not elicit any response. Eating behavior pattern or motor function never return to normal. Subsequent injection associated with repetition of excitement and sedative phases described above; however, the excitement phase is shorter and sedation phase considerably longer (>3 h).

PA). Subdermal electrodes recorded signals from right and left hemispheres representing global hemispheric function rather than localized function obtained from conventional EEG. Baseline CSA recordings were obtained prior to the intrathecal injections of saline or opioids, as well as continuously, after administration of the above agents. Heart rate, respiratory rate, and animal behavior were recorded for comparison with the CSA activity. In 19 sheep CSA was recorded between 30 and 60 min after injection. It was recorded only once for each animal, and the procedure lasted about 1–2 h.

In view of the rapid behavioral changes noted during or soon after injection in some animals, it was decided to study the time course of the spread of drugs from the lumbar injection site to the supraspinal level. In three sheep, a tracer experiment was performed using the radionuclide technetium-diethylene-triamine-pentaacetate (TC-99mDTPA). Chromatographic quality control showed that the radiochemical purity was 99% at the time of injection. The anesthetized animals were positioned on a table on their right side, adjacent to a portable Ohio Nuclear Anger Scintillation camera. The radionuclide was injected intrathecally over a period of 60 s, and the volume of injectate was 5.2 ml, which corresponded to the maximum volume in the large-dose control group. The detector was placed 10 inches from above the animal's head, such that the head and the upper cervical spine were within the field of view. A 140-KeV all-purpose collimator was used with the camera. The images were obtained by collecting 300,000 counts each, over a period of 30 s.[8,9] Markers, consisting of two syringes each containing 50 mCi sodium pertechnetate (Tc-99m) were placed on the skin near the spine. These allowed us to follow the time course of movement of the tracer within the spinal canal. Serial images were obtained during and after injection.

On day 4, behavioral, postural, and motor function of each sheep was evaluated and the animal was killed with intravenous thiopental and potassium chloride. After administration of intravenous heparin, the chest was opened; a large bore plastic tubing was introduced into the right atrium; and exsanguination was permitted through the other end of the tubing. This was followed by the insertion of similar tubing through the left ventricle into the ascending aorta. After the tubing was sutured in position, perfusion was started using Trump's fixative delivered by a peristaltic blood roller pump (Travenol).

A portion of the spinal column that included the spinal cord and meninges was removed carefully. This portion extended from the area of spinal catheter entry to the area 4–5 cm rostral to the tip of spinal catheter. This section of the spinal column including the cord and meninges was immersed in Trump's fixative. Evaluation of gross and microscopic changes was performed by a pathologist blinded to the different drugs. Multiple sections of each spinal cord were processed for light microscopy. The sections were embedded in paraffin, cut at 4–6 μm, and stained with hematoxylin and eosin. Selected sections were stained by silver nitrate and luxol fast blue. Levels corresponding to the catheter insertion, posterior to catheter insertion, catheter tip, and anteriormost segment were examined by a pathologist who was unaware as to the treatment received by the animal. The findings were

scored on a scale of 0 to +++ (0 = no changes; + = mild changes; ++ = moderate changes; and +++ = severe changes). Two animals died, one after receiving butorphanol and the other after sufentanil; for technical reasons, perfusion fixation in these animals was not possible, and therefore their spinal cords were not examined.

Behavioral and motor changes were assessed individually by a blinded person, with each animal serving as its own control. The behavioral and motor changes were also scored on a four-grade scale of 0 to +++ where 0 = no change and +++ = severe changes; see table 2 for details. Changes are presented as mean values and ranges or as percentage changes from baseline values. Statistical analysis was not performed because of the small number of animals in each group.

Results

In eight animals, CSF could not be aspirated from the intrathecal catheters; on dissection it was noted that catheter migration had occurred. The catheter penetrated into the cord in five sheep (two sheep in the nalbuphine group and one each in the butorphanol, sufentanil, and saline groups), which then were excluded from the study. In three sheep (all in the butorphanol group), the catheter migrated into the epidural space. This enabled us to compare the effects of intrathecal administration with epidural administration of butorphanol.

Behavioral and Motor Changes (Table 3)

Butorphanol

During and immediately after administration of butorphanol, five of eight sheep became severely agitated and rigid. Three of these sheep received the large dose and two the small dose. Irrespective of the dose, a consistent sequence of events was noted. Agitation, generalized total body rigidity, frequent distress vocalization (baaing), violent kicking of the forelimbs, tachypnea, and tachycardia were followed by sudden paralyses of the hindlimbs and falling of the animal. These grade-+++ behavioral responses started during injection and lasted 2–3 min. After 20–30 min gradual recovery was noted and was followed by increasing sedation and apathy. The animals appeared uninterested in their surroundings or food.

The motor effects of butorphanol were dose-dependent. In animals receiving the small dose, hindlimb paralysis was prolonged (4–5 h) but motor function recovered spontaneously; however, animals receiving the large doses developed irreversible hindlimb paralysis. Repeated attempts at regular intervals (every 6 h) to help the animal stand were unsuccessful. In this group the hindlimbs remained flaccid, and noxious cutaneous stimulation elicited no response. Bowel and bladder sphincter control was intact. Repeated injections were made only in animals in which complete recovery of motor function was noted.

During the first 15–20 min after injection respiratory rates increased from 15 breaths per min (range 12–19) to 72 breaths per min (range 60–80 breaths per min). During the period of sedation respiratory rates decreased to 10 breaths per min (range 8–13). Subsequent injection of saline in the intrathecal catheters of this group of sheep evoked transient (< 5 min) grade-0 or grade-+ behavioral changes. In three animals no behavioral changes were noted. Two of these animals belonged to the large-dose and one to the small dose groups. On dissection it was found that the intrathecal catheters had migrated epidurally in these sheep. Thus, the behavioral response to butorphanol when administered intrathecally was dramatically different from the response following epidural administration of the drug.

Similar behavioral changes were noted after subsequent injections. In one sheep the behavioral responses described above were followed after about 90 min by deep sedation and severe respiratory depression (cyanosis, respiratory rate 3–4 breaths per min, and carbon dioxide tension 8.8 kPa [66 mmHg]). Repeated intravenous naloxone injections and resuscitation were unsuccessful. The sheep died within 2 h after butorphanol injection (second injection of large dose). Because of the severity of the behavioral responses, prolonged motor paralysis, inability

Table 3. Behavioral and Motor Changes after Intrathecal Administration of Butorphanol, Sufentanil, or Nalbuphine

	Butorphanol		Sufentanil		Nalbuphine		
	Large Dose	Small Dose	Large Dose	Small Dose	Large Dose	Small Dose	Saline
Behavioral changes	+++	+++	++ to +++	+ to ++	+	0	0
Motor impairment	+++	+++	++	+	+*	0	0
Spontaneous recovery of motor impairment	No	Yes†	Yes‡	Yes§	Yes§	—	—
Sedation	++	++	++	+	++	+	0
Mortality	1/6	0/3	1/6	0/3	0/6	0/3	0/5

0 = no change; + = mild; ++ = moderate; +++ = severe.
* Grade + motor impairment was noted in only one animal.
Recovery of motor impairment: †within 4–5 h; ‡within 1.5–3 h; §within 1 h.

to eat or drink for long periods (2–3 h), and death of one animal in this group, it was considered inappropriate to continue with the intrathecal injections of butorphanol every 6 h for 3 days as planned. None of these animals therefore received more than two injections. In the three animals in which epidural migration of the catheter had occurred and that did not demonstrate any behavioral changes, butorphanol was given according to the protocol.

Sufentanil

In contrast to butorphanol the behavioral effects following intrathecal sufentanil were dose-dependent in the doses studied. Intrathecal injection of sufentanil in large doses (7.5 μg/kg) was associated with moderate (grade ++) to severe (grade +++) behavioral changes during and up to 90 s after injection. These effects were similar to those seen after intrathecal butorphanol but lasted 15–20 min, after which the sheep recovered gradually over a period of about 1 h. This was often followed by a period of sedation lasting 2–3 h (table 3). The responses were similar after every injection. Saline injection in these catheters was associated with grade-0 to grade-+ behavioral changes. Generalized rigidity seen after butorphanol was absent in these animals. Intrathecal sufentanil was also associated with dose-dependent hindlimb motor weakness. However, all animals demonstrated spontaneous recovery. Recovery of motor function occurred within 15–30 min after small doses and 1.5–3 h after large doses of sufentanil.

One sheep that received a second injection of a large dose of sufentanil collapsed about 5 min after injection (because of motor paralysis of hindlegs). Apathy, respiratory depression, and sedation were noted within a few minutes of injection. Naloxone partially reversed the sedation, respiratory depression, and motor weakness. Attempts by the animal to stand were unsuccessful. Since the naloxone effect was short-lived, a continuous infusion was started, which improved respiration and muscle tone. However, the animal remained apathetic and refused to eat or drink. Nasogastric feeding was started on the second day. However, on the third day the animal died because of severe respiratory depression, despite the treatment with naloxone.

Small doses (1.5 μg/kg) of intrathecal sufentanil evoked similar behavioral responses, but the changes were mild (grade +) to moderate (grade ++). Hindlimb motor weakness was also mild (grade +); spontaneous recovery was noted by 30 min. These animals recovered after 15–30 min and could eat and drink normally (table 3).

Nalbuphine

Except for moderate sedation and grade-0 to grade-+ behavioral changes, no other changes were noted in four sheep receiving the large dose (0.75 mg/kg) or in the two sheep receiving the small dose (0.15 mg/kg) of intrathecal nalbuphine. In one animal, hindlimb motor weakness occurred 5–10 min after intrathecal injection of large doses of nalbuphine (0.75 mg/kg), and the animal was unable to stand. Complete recovery of motor function was noted within 60 min of injection. Motor function returned within 30–60 min (table 3).

Saline

No behavioral or motor changes were seen in animals receiving intrathecal saline injections every 6 h during the 3-day study period (table 3).

EEG Changes

High-amplitude and/or high-frequency EEG activity lasting 20–30 min was seen in all animals receiving butorphanol and in those receiving large doses of sufentanil. This was followed by normalization and subsequent decreased cortical activity for 2–3 h. In contrast, low-amplitude and/or low-frequency EEG activity due to sedation was seen in animals receiving small doses of sufentanil and in those receiving nalbuphine. This activity was not dose-dependent after intrathecal nalbuphine. Intrathecal saline in control animals did not evoke any EEG changes. Increased cortical activity or seizure activity was noted during and immediately after injection in all five animals (all groups) where the intrathecal catheter was subsequently shown to have penetrated into the spinal cord.

Histologic Changes (Table 4)

Butorphanol

Histopathologic changes following intrathecal administration of butorphanol consisted of moderate to severe (grade-++ to grade-+++) inflammatory changes. The changes were more severe after large doses; in these animals, changes suggestive of suppurative meningitis and myelitis were noted (figs. 1 and 2). Neuronal changes such as spongiosis and chromatolysis of a mild nature (grade +) and axonal changes of moderate nature (grade ++) were noted after large doses of butorphanol. These changes were less severe after small doses of butorphanol (table 4).

Sufentanil

Intrathecal administration of the small dose of sufentanil (1.5 μg/kg) was associated with mild inflammatory reaction (grade +), some axonal swelling, and minor neuronal changes manifested as shrunken neurons. Larger doses (7.5 μg/kg), however, were associated with moderate (grade ++) to severe (grade +++) inflammatory

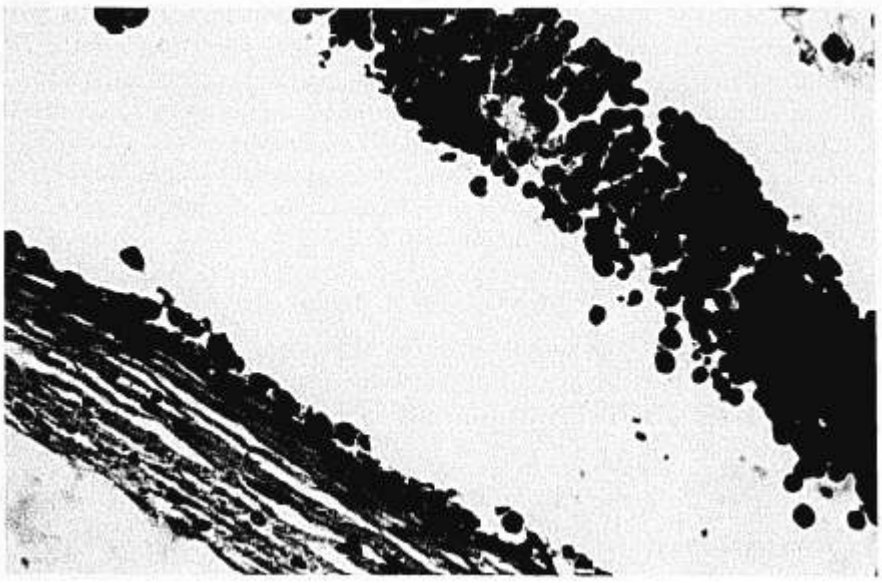

FIG. 1. Section at level of catheter tip. Inflammatory changes suggestive of suppurative meningitis due to irritating effect of small dose (0.075 mg/kg) of intrathecal butorphanol (Electron microscopy ×400).

changes (meningitis and arachnoiditis) and grade + chromatolysis of neurons as well as axonal swelling (fig. 3). In general, the histopathologic changes following intrathecal sufentanil were not as severe as those following butorphanol (table 4).

Nalbuphine

Mild inflammatory changes (grade +) were noted irrespective of the dose of nalbuphine. However, in the large-dose group, grade + neuronal changes were also noted (table 4).

Saline (Control)

In four of the five sheep receiving saline, the catheter was found to be correctly placed. No pathologic changes in the nerve roots, neurons, or axons were noted in these animals. In two of these four sheep there was evidence of mild suppurative meningitis (grade +) at the level of catheter insertion and catheter tip.

SHEEP TRACER EXPERIMENT

Injection of TC-99mDTPA into intrathecal catheters showed that the time course of movement of the tracer

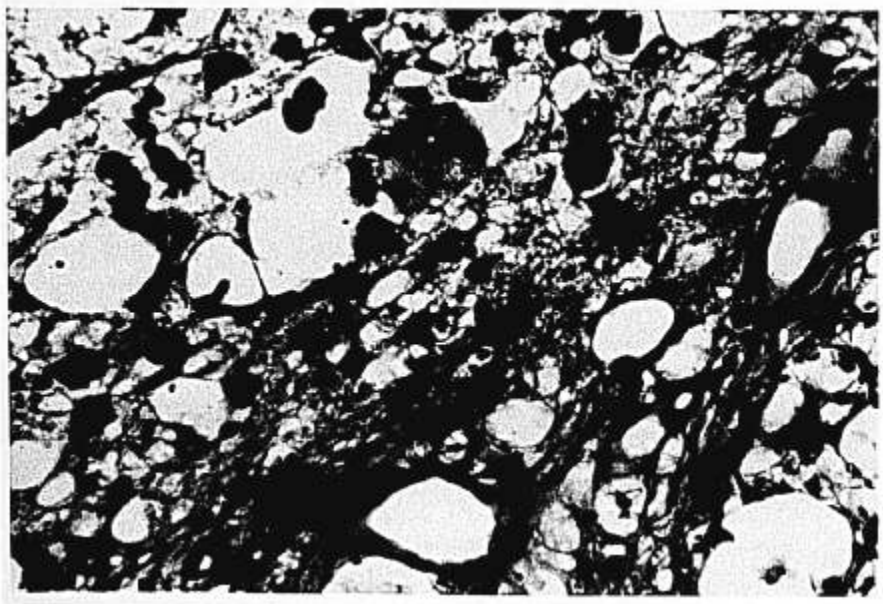

FIG. 2. Axonal and neuronal degeneration following small dose intrathecal butorphanol. Hemorrhages near catheter insertion: section at level of catheter insertion (electron microscopy ×400).

TABLE 4. Spinal Cord Histologic Changes Following Intrathecal Administration of Butorphanol, Sufentanil, or Nalbuphine

	Butorphanol*		Sufentanil		Nalbuphine		
	Large Dose	Small Dose	Large Dose	Small Dose	Large Dose	Small Dose	Saline
Inflammatory changes	+++	+++	++ to +++	+	+	+	+†
Neuronal changes	+	+	+	+	+	0	0
Axonal changes	++	+	+	+	0	0	0

Histopathologic changes: 0 = no change; + = mild; ++ = moderate; +++ = severe.

* No histopathologic changes in three sheep were subsequent examination showed epidural migration of catheter.

† The inflammatory changes were noted in only two sheep.

was variable. Movement of radioactivity from the site of injection in the lumbar area to the ear level of the three sheep was noted at 6, 30, and 60 min respectively. The fastest movement of radioactivity was at the rate of 15 cm/min.

Discussion

As we gain more experience with epidural and intrathecal opioids, it has become evident that several agents may play a role in the management of pain by this modality. In the literature, morphine has been the most widely used drug for epidural or intrathecal administration.[1-4] Animal studies in different species have demonstrated that intrathecal administration of morphine is not associated with spinal cord histopathologic changes.[10,11] However, it is believed by many workers that morphine may not be the ideal drug and that other opioids, particularly highly lipophilic ones, may be safer because of the presumed lack of late onset respiratory depression.[1-4,12] This has resulted in the trial of a large number of opioids and nonopioids. Thus, more than 20 different drugs have been administered epidurally; however, surprisingly few animal studies have preceded their clinical application.[4-7] Epidural administration of sufentanil, butorphanol, or nalbuphine in humans has been shown to provide satisfactory postoperative analgesia, and no neurotoxic effects have been reported so far.[13-15] However, many workers believe that animal neurotoxicity studies are necessary before use of these potentially attractive opioids.[5-7]

It has been suggested that morphologic investigation without functional studies may not be adequate to detect neurotoxic potential of spinally administered drugs. Thus, the absence of morphologic changes is not alone sufficient to free a drug from possible neurotoxic effects.[7] Conversely, it is possible for behavioral effects not to be observed and toxicity still to be present. Therefore, in the present study in a sheep model, histopathologic changes as well as behavioral, motor, and EEG changes were evaluated. Sheep were chosen because of their large size and relatively easy handling. The sheep model has been used by others to study neurotoxicity of intrathecal local

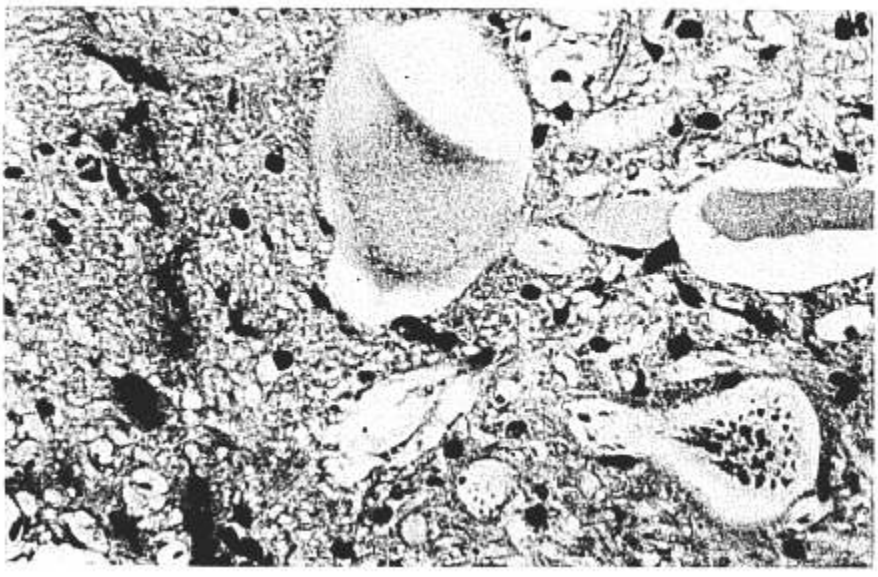

FIG. 3. Section at the level of catheter insertion showing chromatolysis. This animal received intrathecal sufentanil 7.5 μg/kg (electron microscopy ×400).

anesthetics[16] and antinociception following epidural clonidine.[17] Of the three opioids studied, butorphanol was clearly the most irritating to the nervous tissue.

Irrespective of dosage, all animals receiving intrathecal butorphanol showed severe behavioral changes. They also demonstrated evidence of increased cortical activity. In addition, the animals developed irreversible hindlimb paralysis after large doses (0.375 mg/kg), and severe motor weakness lasting 4–5 h after small doses (0.075 mg/kg) of intrathecal butorphanol. One animal died because of severe respiratory depression that was unresponsive to repeated doses of naloxone. The histopathologic changes were also most severe in the group of animals receiving intrathecal butorphanol. The histology was dose-dependent and consisted of moderate to marked inflammatory cell reaction, suppurative meningitis, and myelitis.

It was interesting that the doses of butorphanol that were highly neurotoxic when administered intrathecally appeared harmless after epidural administration, thus demonstrating the efficacy of dura as a remarkable barrier to the deleterious effects of butorphanol. The epidural space is believed to be relatively tolerant to abuse. Solutions such as thiopental, methohexital, diazepam, potassium chloride, magnesium sulfate, total parenteral nutrition solution, collodion, cephazolin, and hypertonic saline have been accidentally administered in the epidural space with only minor or transient symptoms.[18,19] However, permanent paraplegia occurred after epidural administration of collodion, hypertonic saline, and 11.25% potassium chloride.[20,21] Thus, the lack of neurotoxicity in the few clinical reports of epidural butorphanol administration may not be valid if the drug is administered intrathecally by design or by accident.

In contrast to butorphanol the behavioral and motor changes due to sufentanil were dose-dependent. When the drug was administered in large doses the changes were generally similar to those described for butorphanol. In this group also one animal died because of severe respiratory depression that did not respond to repeated injections of intravenous naloxone. Large doses of intrathecal sufentanil have been associated with similar responses in other species[22]: motor dysfunction and catalepsy were noted in all rats receiving 10 or 30 μg intrathecal sufentanil; furthermore, a mortality of 20% and 60% respectively was noted. Similarly, following 100 μg intrathecal sufentanil, cats became excited and displayed labored breathing and hindlimb dysfunction. After 10–15 min the animals became quiet and behaviorally depressed, and motor weakness disappeared within 2 h. Extremely large dose of intrathecal sufentanil (300 μg) resulted in convulsions, labored breathing, and death after 7 h.[22] However, other than inflammatory reactions secondary to catheter placement no abnormal spinal cord pathology was noted in cats in spite of extremely large doses (ED_{100} and $10 \times ED_{100}$) of sufentanil.[22] In our study too, catheter-related inflammatory reaction and lack of spinal cord pathology were noted after small doses (about 40 μg) of sufentanil.

However, in our study large doses (120 μg) of sufentanil, although well below the histopathologically safe doses demonstrated in cats,[22] nevertheless were associated with meningitis, arachnoiditis, chromatolysis of neurons, and axonal swelling, suggesting that sufentanil neurotoxicity may be species-related. When administered epidurally, even large doses of sufentanil appeared harmless. In the literature, sufentanil in dosages as great as 50-μg boluses combined with 25 μg/h has been used for management of labor pain and postoperative pain without any reports of neurotoxicity.[23,24] Furthermore, dosages as large as 600–800 μg/day have been administered epidurally for periods of weeks to control cancer pain, and no evidence of spinal cord histopathologic changes was noted.**

Nalbuphine was clearly the least irritating to neural tissue. Large doses of intrathecal nalbuphine (about 20 mg) were associated with relatively minor behavioral and EEG changes. Mild motor impairment was seen in only one animal; it lasted 60 min. Sedation was a common feature in these animals. None of the animals receiving nalbuphine died. Histology of the spinal cord showed mild inflammatory and neuronal changes. Except for sedation and mild respiratory depression, the behavioral, motor, and histopathologic changes following small doses of intrathecal nalbuphine (about 4 mg) were generally similar to those seen in control animals. Thus, the order of severity of behavioral, motor, and histopathologic changes in our study was butorphanol > sufentanil > nalbuphine.

A surprisingly large number of catheter migrations were noted on dissection. In three animals the catheters migrated epidurally, and in five they penetrated the spinal cord. Indentation rather than penetration of the cord is generally seen in cats and dogs receiving intrathecal catheters.†† Cord penetration by intrathecal catheters may be related to exaggerated back movements due to the intense irritation by intrathecal opioids and to anatomic features of the sheep spinal cord and intrathecal space; however, this does not explain epidural migration. The largest number of catheter migrations occurred in the animals receiving butorphanol, which was the opioid most irritating to the neural tissue. Possible contributing factors

** Boersma FP, Noorduin H, Vanden Bussche G: Epidural sufentanil for cancer pain control in outpatients. Presented at the VIII Annual Meeting of the European Society of Regional Anaesthesia. Lisbon, Portugal, May 1989.

†† Yaksh T: Personal communication.

may have been the small amount of lumbar CSF (about 15 ml) or more likely the Portex catheter, which has a larger diameter and is stiffer than the catheters (PE-50 tubing) used by others.‡‡ Nevertheless, spinal cord penetration by a PE-90 intrathecal catheter in a dog model was reported in a recent study.[25] However, these unexpected catheter migrations permitted us to compare the effects of intrathecal *versus* epidural butorphanol as well as to compare the effects of injection of the three opioids and saline into the spinal cord. Although the time of epidural catheter migration was unclear in our study, we believe that it occurred prior to drug administration because of our inability to aspirate CSF and the consistent lack of behavioral and motor responses in this group. As can be expected, the intraneural injection of the three opioids and saline was associated with motor weakness and exaggerated behavioral responses suggestive of intense pain and neural irritation.

It has been suggested that an acute increase in CSF volume by subarachnoid administration of a relatively large volume of drug could raise CSF pressure to such an extent as to reduce spinal cord blood flow and cause neurologic deficit.[16] Rosen *et al.* demonstrated both neurologic deficits and/or histologic changes by injecting 10 ml of three different local anesthetics into the subarachnoid space of sheep, but also demonstrated similar changes with lumbar puncture alone.[16] The spinal cord extends to the lower sacrum in sheep and the volume of lumbar CSF in the spinal canal is relatively low, estimated to be about 15 ml.[16,26] It is conceivable that some behavioral changes, especially in animals receiving large doses of opioids in our study, may have resulted from the relatively large volumes of injectate. However, the largest volume injected in our study was 5.2 ml. This is well below volumes (10 ml) injected by Rosen *et al.*[16] In our study there was a dramatic difference in the behavioral responses between the animals receiving the large but identical volumes of saline or butorphanol. When an identical volume of saline was injected intrathecally in animals who had recovered from severe behavioral effects of large doses of butorphanol, no behavioral changes occurred. These findings suggest that the behavioral and neurologic changes in our study were due predominantly to the drug itself rather than barotrauma due to relatively large volumes of injectate.

Neurotoxicity appears to be related to local acute concentration and the duration of the sustained levels. Because of the very small intrathecal space in sheep, the acute volume of distribution in this animal may be very small in contrast to that in humans. In humans the concentration of injectate after intrathecal injection is diluted considerably after 5–10 min, whereas in sheep this dilution is not possible. Furthermore, the large volume administered will prevent the injected drug from being diluted by the CSF. It should be noted that in a considerable number of animals the behavioral, motor, respiratory, and EEG changes were seen during or shortly after injection of drugs. Due to the small volume of sheep spinal CSF, the injected opioids are apparently squeezed rostrally by barbotage effect of intrathecal injection. Our TC-99mDTPA tracer experiment showed that movement of radioactivity from lumbar site of injection to ear level may take as little as 6 min. Rapid transport of intrathecally administered drugs from lumbar spinal to supraspinal levels has been reported in animal and human studies.[22,27] However, the behavioral, motor, and EEG changes in the present study may have been due to spinal as well as supraspinal actions of the opioids.

Although equivalent intrathecal analgesic doses of butorphanol, sufentanil, or nalbuphine are not available, studies with local anesthetics have indicated that sheep probably have milligram-per-kilogram dose requirements similar to those of humans.[28] Therefore, the sheep we studied received doses that would ordinarily be used epidurally in humans (small dose). To exaggerate neurotoxic effects, some animals were given five times this amount (large dose). Our protocol for drug administration, for only 4 days instead of 7–14 days, may be questioned. However, instead of the more usual single daily injection we administered the drugs every 6 h because this closely parallels their use in clinical routine and also because of the reported short duration of analgesia of these opioids.[13–15,23,24] In the present study commercial solutions of butorphanol and sufentanil were used; only the nalbuphine solution was prepared from pure powder. Commercially available sufentanil and butorphanol are preservative-free, whereas nalbuphine contains sodium metabisulfite in addition to methylparaben and propylparaben as preservatives. Therefore, in the present study, nalbuphine was administered as a freshly prepared solution by dissolving the powder in saline prior to injection. However, it should be noted that all three opioids contain citrate and that butorphanol also contains tartrate salt. The manufacturers of the sufentanil we used do not recommend the commercially available drug for epidural or intrathecal administration in humans; they expect to introduce a *p*H-adjusted formulation of sufentanil specially for epidural and intrathecal administration.§§ Although none of the opioids contained preservatives, the role of injected vehicles is unclear in our study.

‡‡ Yaksh T: Personal communication.

§§Henk Noorduin, personal communication, Janssen Pharmaceuticals, Belgium.

In conclusion, our results suggest that intrathecal injection of butorphanol or of large doses of sufentanil are associated with severe behavioral, motor, EEG, and spinal cord histologic changes and may have neurotoxic potential in humans. Large and small doses of nalbuphine and small doses of sufentanil that correspond to therapeutic epidural doses in humans appear to be relatively safe when injected intrathecally. There was a general correspondence between the degree of behavior change and the degree of histologic change. Our study also shows that a drug that may exhibit safety in the epidural space may not be safe intrathecally. Further studies in other animal species and carefully controlled clinical studies are necessary before butorphanol or large doses of sufentanil can be recommended for intrathecal administration in humans.

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References

1. Morgan M: The rational use of intrathecal and extradural opioids. Br J Anaesth 63:165–188, 1989
2. Cousins MJ, Mather LE: Intrathecal and epidural administration of opioids. ANESTHESIOLOGY 61:276–310, 1984
3. Etches RC, Sandler AN, Daley MD: Respiratory depression and spinal opioids. Can J Anaesth 36:165–185, 1989
4. Rawal N: Indications for the use of intraspinal opioids, Spinal Narcotics. Edited by Rawal N and Coombs DW. Boston, Kluwer Academic Publications, 1990, pp 43–62
5. Eisenach JC: Demonstrating safety of subarachnoid calcitonin: Patients or animals? Anesth Analg 67:298, 1988
6. Yaksh TL, Collins JG: Studies in animals should precede human use of spinally administered drugs. ANESTHESIOLOGY 70:4–6, 1989
7. Gordh Jr T, Post C, Olsson Y: Evaluation of the toxicity of subarachnoid clonidine, guanfacine, and a substance P-antagonist on rat spinal cord and nerve roots: Light and electron microscopic observations after chronic intrathecal administration. Anesth Analg 65:1303–1311, 1986
8. Kim EE, Haynie TP: Central nervous system imaging, Nuclear Diagnostic Imaging. Edited by Kim E. Haynie T. New York, MacMillan Publishing Co., 1987, pp 444–456
9. Staab EV: Radionuclide cisternography, Clinical Radionuclide Imaging. Edited by Freeman LM. Orlando, Grune and Stratton, 1984, pp 679–703
10. Abouleish E, Barmada MA, Nemoto EM, Tung A, Winter P: Acute and chronic effects of intrathecal morphine in monkeys. Br J Anaesth 53:1027–1032, 1981
11. King FG, Baxter AD, Mathieson G: Tissue reaction of morphine applied to the epidural space of dogs. Can Anaesth Soc J 31: 268–271, 1984
12. Rawal N: Adverse effects of spinal opioids in acute pain and their management, Spinal Narcotics. Edited by Rawal N, Coombs DW. Boston, Kluwer Academic Publications, 1990, pp 77–88
13. Abboud TK, Moore M, Zhu J, Murakawa K, Minehart M, Longhitono M, Terrasi J, Klepper ID, Choi Y, Kimball S, Chu G: Epidural butorphanol or morphine for relief of post-cesarean section pain: Ventilatory responses to carbon diozide. Anesth Analg 66:887–893, 1987
14. Donadoni R, Rolly G, Noorduin H, Vanden Bussche G: Epidural sufentanil for postoperative pain relief. Anaesthesia 40:634–638, 1985
15. Wang JJ, Mok MS, Lippmann M: Comparative analgesic efficacy of epidural nalbuphine, butorphanol, meperidine and morphine (abstract). Anesth Analg 67:S248, 1988
16. Rosen MA, Baysinger CL, Shnider SM, Dailey PA, Norton M, Curtis JD, Collins M, Davis RL: Evaluation of neurotoxicity after subarachnoid injection of large volume of local anesthetic solutions. Anesth Analg 62:802–808, 1983
17. Eisenach JC, Dewan DM, Rose JC, Angelo JM: Epidural clonidine produces antinociception, but not hypotension, in sheep. ANESTHESIOLOGY 66:496–501, 1987
18. Wells D, Davies G, Wagner D: Accidental injection of epidural methohexital. ANESTHESIOLOGY 67:846–848, 1987
19. Kopacz DJ, Slover RB: Accidental epidural cephazolin injection: Safeguards for patient-controlled analgesia. ANESTHESIOLOGY 72:944–947, 1990
20. Shanker KB, Palkar NV, Nishkala R: Paraplegia following epidural potassium chloride. Anaesthesia 40:45–47, 1985
21. Usubiaga JE: Neurological complications following epidural analgesia. Int Anesthesiol Clin 13:19–50, 1975
22. Yaksh TL, Noueihed RY, Durant PAC: Studies of the pharmacology and pathology of intrathecally administered 4-anilinopiperidine analogues and morphine in the rat and cat. ANESTHESIOLOGY 64:54–66, 1986
23. Powell GM, Steinberg RB: Epidural sufentanil alone for analgesia for labor and delivery (abstract). Anesth Analg 68:S226, 1989
24. Madej TH, Watson NC, Martin BC: Large dose epidural sufentanil. Management of postoperative pain with few side effects. Anaesthesia 42:1204–1206, 1987
25. Coombs DW, Colburn RW, Allen CD, Deroo DB, Fratkin JD. Toxicity of chronic spinal analgesia in a canine model: Neuropathologic observations with dezocine lactate. Reg Anesth 1990;15:94–102
26. Payne R, Madsen J, Harvey RC, Inturrisi CE: A chronic sheep preparation for the study of drug pharmacokinetics in spinal and ventricular CSF. J Pharmacol Methods 16:277–296, 1986
27. Drayer BP, Rosenbaum AE: Studies of the third circulation. Amipaque CT cisternography and ventriculography. J Neurosurg 48:946–956, 1978
28. Wallis KL, Shnider SM, Hicks JS, Spirey HT: Epidural anesthesia in the normotensive pregnant ewe: Effects on uterine blood flow and fetal acid–base status. ANESTHESIOLOGY 44:481–487, 1976