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Intravenous Opioids Stimulate Norepinephrine and Acetylcholine Release in Spinal Cord Dorsal Horn

Systematic Studies in Sheep and an Observation in a Human

Hervé Bouaziz, M.D., Chuanyao Tong, M.D.,† Young Yoon, M.D.,* David D. Hood, M.D.,‡ James C. Eisenach, M.D.§*

Background: **Opioids produce analgesia by direct effects as well as by activating neural pathways that release nonopioid transmitters. This study tested whether systematically administered opioids activate descending spinal noradrenergic and cholinergic pathways.**

Methods: **The effect of intravenous morphine on cerebrospinal fluid and dorsal horn microdialysate concentrations of norepinephrine and acetylcholine was examined in 20 sheep. Animals received either intravenous morphine or fentanyl alone, or morphine plus intravenous naloxone or intrathecal idazoxan.**

Results: **Intravenous morphine (0, 0.5, 1 mg/kg, intravenous) produced dose-dependent increases in cerebrospinal fluid norepinephrine and acetylcholine, but not epinephrine or dopamine. Morphine's effect was blocked by intravenous naloxone and by intrathecal idazoxan. In microdialysis experiments, intravenous morphine increased the concentration of norepinephrine and acetylcholine, but not epinephrine or dopamine, in the dorsal horn. In contrast, intravenous morphine exerted no effect on any of these monoamines in the ventral horn. Intravenous naloxone and cervical cord transection each blocked morphine's effect on dorsal horn norepinephrine.**

Conclusions: **These results support functional studies that indicate that systematically administered opioids cause spinal norepinephrine and acetylcholine release by a naloxone-sensitive mechanism. Idazoxan blockade of morphine's effects on cerebrospinal fluid norepinephrine was unexpected, and suggests that both norepinephrine and acetylcholine release in the spinal cord may be regulated by α_2-adrenoceptors. Microdialysis experiments suggest increased norepinephrine and acetylcholine levels in cerebrospinal fluid resulted from intravenous morphine-induced activation of bulbospinal pathways. (Key words: Analgesics, opioid: morphine. Effect site: spinal cord. Cerebrospinal fluid components: acetylcholine; norepinephrine.)**

OPIOIDS, which remain the mainstay for the treatment of moderate to severe pain, cause analgesia by actions at several interrelated sites. Opioids have direct effects in the periphery,[1] the brain,[2] and the spinal cord[3] to cause analgesia. In addition, a variety of interactions among these sites has been described. For example, opioids can activate vagal afferents in the periphery to cause a centrally mediated analgesia,[4] and opioid actions in the brain and the spinal cord interact in a synergistic manner.[5] An important mechanism of systemically administered opioids in causing analgesia is activation of neurons in the mid-brain and medulla with descending inhibitory projections to the spinal cord dorsal horn.[6] Chief among inhibitory neurotransmitters released is norepinephrine, which diminishes substance P release from primary Aδ and C afferents,[7] and reduces response of dorsal horn neurons to noxious stimulation.[8]

Whereas there are many studies supporting the relevance of descending spinal noradrenergic inhibition to analgesia from systemically administered opioids, most focus on anatomic studies,[9] electrophysiologic recordings,[8] or behavioral studies of analgesia and intrathecal injection of noradrenergic antagonists.[10] There remains little direct evidence of spinal norepinephrine release caused by systemically administered opioids in the nonanesthetized whole animal. Similarly, although systemically administered opioids cause an-

* Visiting Scholar to the Department of Anesthesia.

† Research Assistant Professor of Anesthesia.

‡ Associate Professor of Anesthesia.

§ Professor of Anesthesia.

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Address reprint requests to Dr. Eisenach: Department of Anesthesia, Wake Forest University Medical Center, Medical Center Boulevard, Winston-Salem, North Carolina 27157-1009. Address electronic mail to: eisenach@isnet.is.wfu.edu.

algesia that can be antagonized by intrathecal atropine,[11,12] there are no studies examining the relationship between intravenous opioid administration and cerebrospinal fluid (CSF) norepinephrine and acetylcholine.

The above studies suggest that systemic opioids cause norepinephrine and acetylcholine release in the spinal cord. Other studies suggest that these neurotransmitters are linked, such that spinally released norepinephrine directly causes acetylcholine release by actions on α_2-adrenoceptors.[13] Thus, intravenous morphine may induce release of norepinephrine in the spinal cord, which acts on excitatory α_2-adrenoceptors on cholinergic neurons. The purpose of the current study was to investigate the effect of opioid stimulation by intravenous morphine in nonanesthetized sheep on CSF concentrations of norepinephrine and acetylcholine and to determine whether norepinephrine and acetylcholine release were linked by α_2-adrenoceptor stimulation. The source of changes in neurotransmitter concentrations in CSF was further examined using microdialysis, including comparisons of dorsal *versus* ventral horn, norepinephrine *versus* other monoamines, and intact *versus* transected spinal cord. Finally, the effect of intravenous morphine on CSF norepinephrine and acetylcholine was determined in one volunteer in whom CSF could be repeatedly sampled *via* an indwelling spinal catheter.

Materials and Methods

Cerebrospinal Fluid Sampling in Sheep

After Animal Care and Use Committee approval, 10 ewes of mixed Western breeds were studied. After a 24-h fast, anesthesia was induced by intravenous injection of 5–10 mg/kg ketamine and 10–15 mg/kg pentobarbital, the trachea was intubated, and anesthesia was maintained with 1–2% halothane *via* mechanical ventilation.

Polyvinyl catheters were inserted under direct vision into a femoral artery and vein and advanced 15 cm centrally. The catheters were tunneled subcutaneously to the flank, where they were maintained in a canvas pouch, and the groin incision closed. The animal was turned prone and a hemilaminectomy was performed over a lower lumbar interspace. The dura mater was exposed, a small hole was made, and a 21-G polyethylene catheter was advanced 5–8 cm cephalad in the upper lumbar intrathecal space. The catheter was secured by retention sutures, the incision was closed, and the animal was allowed to awaken from anesthesia. Antibiotic prophylaxis was provided with 500,000 units intramuscular penicillin G daily through the second postoperative day. Evidence of postoperative pain was to be treated with 1.1 mg/kg intramuscular flunixin. Animals were examined every 15 min until standing, then every 30 min until eating, then twice daily by an animal technician blinded to the study and certified through our institutional training for personnel caring for animals. Typical pain behaviors in individually housed sheep include lethargy, recumbency, head turning toward wound, and retraction of lips. In no case was behavioral evidence of postoperative pain observed. At least 3 days passed from surgery to experiments.

On the day of the experiment the intrathecal and intravenous catheters were tested for correct location by withdrawal of CSF and blood, respectively. Each animal then received, in random order and with experiments separated by a minimum of 72 h, saline or morphine, 0.5 or 1.0 mg/kg by rapid intravenous bolus. Cerebrospinal fluid samples (1 ml) were obtained before and at 10-min intervals for 30 min, then at 60 min after intravenous injection. One half of the sample was collected into a tube containing ethylene diamine tetraacetic acid for catecholamine analysis and the other half collected into a tube containing physostigmine for acetylcholine analysis. Both samples were immediately frozen on powdered dry ice and kept at −70°C until analysis. To determine whether morphine was affecting CSF neurotransmitter concentrations by actions on opioid receptors, five animals received 1.0 mg/kg intravenous naloxone, 5 min before 1.0 mg/kg intravenous morphine. To determine whether morphine-induced increases in CSF norepinephrine caused increased CSF acetylcholine *via* activation of α_2-adrenoceptors, five other animals received the specific α_2-adrenergic antagonist, idazoxan, 1.0 mg intrathecally 5 min before 1.0 mg/kg intravenous morphine. Because norepinephrine produces its antinociceptive effects in the spinal cord *via* α_2-adrenoceptor stimulation, we postulated that idazoxan would inhibit norepinephrine-induced increased acetylcholine.

Spinal Cord Microdialysis in Sheep

Surgical Preparation. After approval from the Animal Care and Use Committee, a total of ten ewes of mixed Western breeds, weighing between 45 and 60 kg, were studied. After a 24-h fast, anesthesia was in-

duced with 20–30 mg/kg intramuscular ketamine, and maintained with 0.5% inhalational halothane throughout the entire microdialysis experiment. Muscle relaxation was maintained with incremental intravenous pancuronium. End-tidal CO_2 was continuously monitored and arterial blood was sampled intermittently for blood gas and pH analysis. Blood pressure and heart rate were intermittently monitored *via* a femoral artery catheter, and drugs were administered intravenous *via* a jugular venous catheter.

The animal was turned prone and a three-level, bilateral laminectomy performed in the upper lumbar region, leaving the dura mater and portions of the dorsal spinous processes intact for stability. Two to eight microdialysis probes were inserted transversely through the dorsal horns and, in some cases, the ventral horns, at different sites along the exposed spinal cord. After completion of the experiment, correct anatomic location of probe insertion was verified in each case by dissection. In approximately one of six probes, the area of active dialysis membrane was examined by perfusion of the probes with methylene blue dye, cryosection, and microscopic examination.

Microdialysis Procedure. Microdialysis probes were prepared not more than 12 h before the surgical procedure using hollow fiber bundles (Spectrum, Houston, TX) with an internal diameter of 150 μm and a molecular weight cutoff of 9000 d. The window of active membrane for exchange was precisely defined using two pieces of silica tubing (SGE Inc., Austin, TX), which were inserted through each end of the hollow fiber and advanced so that the tips of each silica tube were separated by exactly 5 mm, corresponding to the length necessary to cover the two dorsal horns of the spinal cord (observation based on fixation of the lumbar spinal cord of several sheep for anatomic examination). The junctions between the silica tubing and the hollow dialysis fiber were sealed using acrylic glue. A wire, 0.035 mm external diameter, (Fisher Scientific, Pittsburgh, PA) was inserted and sealed on one end of the probe and the free end sharpened, thereby allowing penetration of the probe through the dura mater and cord while minimizing tissue damage. After insertion, the portion of the silica tubing connected to the wire was cut and removed in to allow perfusion.

The inlet of the probe was continuously perfused *via* a pump at a rate of 2 μl/min with artificial CSF of the following composition: 145 mM NaCl, 27 mM KCl, 10 mM $MgCl_2$, 12 mM $CaCl_2$, 20 mM Na_2HPO_4 in filtered deionized water. This perfusion rate was chosen based on *in vitro* recovery rates for norepinephrine (10^{-8} M) of 27% with 1 μl/min, 22% with 2 μl/min, and 17% with 3 μl/min.

Experimental Procedure. Fifteen-minute microdialysis samples were collected into small vials on ice, resulting in 30-μl sample volumes. The first 90 min of perfusion were considered as the washout period, allowing for tissue recovery, and were followed by a 60 min collection (4 samples) for baseline. After this initial period, different sets of experiments were performed as follow. In all cases, samples were collected every 15 min for 2 h after opioid administration, and experiments began a minimum of 3.5 h from the time of administration of ketamine for induction of anesthesia.

1. To test the effects of intravenous opioids on monoamine concentrations, either 10 μg/kg fentanyl, (3 sheep) or 1 mg/kg morphine, (3 sheep) was injected intravenous as a bolus (10 ml volume). Acetylcholine concentrations were determined only in sheep receiving 1 mg/kg morphine.
2. To test the opioid receptor mediation of the effects of fentanyl and morphine on catecholamine concentrations, the opioid receptor antagonist, 1 mg/kg naloxone, was injected intravenously 15 min before fentanyl (2 sheep) or morphine (1 sheep).
3. To test the dependence of supraspinal-spinal pathways on the effects of intravenous opioids, a complete transection of the cervical spinal cord was performed in one sheep immediately after insertion of the microdialysis probes, and intravenous morphine was administered 90 min later.

Human Study

One of the authors (JCE) was a volunteer in a study examining the safety of spinally administered neostigmine for analgesia.[14] Institutional Review Board approval was granted, in this one case only, of extension of the study to examine intravenous morphine. At the completion of the neostigmine study, 24 h after spinal neostigmine, 50 μg had been injected, the volunteer received 10 mg intravenous morphine, and CSF was sampled *via* an indwelling lumbar spinal catheter at 10, 20, 30, 45, 60, 90, and 120 min after morphine injection. At these same times, resting end-tidal CO_2 by capnography was measured. In addition, pain report to immersion of the foot in stirred ice water for 60 s by a 10-cm visual analog scale anchored at no pain and

Table 1. Baseline Cerebrospinal Fluid Concentrations of Neurotransmitters

Treatment Group	Acetylcholine	Norepinephrine	Epinephrine	Dopamine
Saline control	0 (0–0)	0.36 (0.29–0.69)	0.33 (0.10–0.48)	0.099 (0.057–0.16)
Morphine, 0.5 mg/kg	0 (0–0)	0.47 (0.38–0.65)	0.29 (0.04–0.32)	0.012 (0–0.13)
Morphine, 1 mg/kg	0 (0–18)	0.65 (0.44–1.0)	0.17 (0.06–0.53)	0.045 (0–0.13)
Morphine + idazoxan	0 (0–14)	0.48 (0.31–0.50)	0.10 (0–0.32)	0.009 (0–0.15)
Morphine + naloxone	0 (0–0)	0.42 (0.19–0.91)	0.59 (0.07–1.2)	0.16 (0.07–0.44)

Neurotransmitter concentrations in pmol/ml. Each value represents the median (25th–75th percentile) of 5 to 10 animals. There were no significant differences.

worst imaginable pain was measured before and 30 min after morphine injection, although the volunteer was not blinded to the drug (morphine) or the time of its injection.

Neurochemical Assays. Samples were stored at −70°C until analysis. Catecholamine concentrations were determined, after alumina extraction, by high-pressure liquid chromatography with electrochemical detection. This method has an interassay coefficient of variation of <9% for norepinephrine, epinephrine, and dopamine, and an absolute detection limit of 12 fmol for norepinephrine, 3.3 fmol for epinephrine, and 1.8 fmol for dopamine.[15] Acetylcholine concentrations were determined by a different high-pressure liquid chromatographic-electrochemical detection method, using other equipment than that for catecholamines. This method has an interassay coefficient of variation of 8% and a detection limit of 50 fmol.[13] In control experiments, addition of morphine to standard solutions did not shift the retention times or alter the peaks for monoamines or acetylcholine.

Drugs used in this study were halothane, ketamine, pentobarbital, and penicillin G (Barber Veterinary Supply, Richmond, VA), idazoxan (donated by Reckitt and Colman, Newcastle-Upon-Tyne, UK), morphine hydrochloride (Abbott Laboratories, Chicago, IL), and naloxone hydrochloride (Sigma Chemical Co., St Louis, MO).

Data Analysis. Unlike the remaining data, CSF concentration data in sheep were not all normally distributed, even after log transformation, so are presented as median + 75th percentiles. Effect of drug treatment over time on CSF neurotransmitter concentrations was determined within each treatment by one-way analysis of variance (ANOVA) for repeated measures followed by Dunnett's test or, if not normally distributed, by Freidman's ANOVA followed by Dunn's test. Treatments were compared for peak CSF neurotransmitter concentrations by one-way ANOVA or, if not normally distributed, by Kruskal-Wallis test, followed by Newman-Keuls test. Within each treatment, baseline concentrations of neurotransmitters were compared to the peak concentrations in the first 30 min after morphine injection by Student's *t* test or, if not normally distributed, by Mann-Whitney *U* test, with Bonferroni correction for multiple comparisons.

For microdialysis experiments, the four 15-min baseline samples for each probe did not differ in concentration of catecholamines by one-way ANOVA, and, for each probe, the average value of these four samples was used as the baseline control. Thereafter, paired 15-min samples were averaged for each 30-min period to decrease variability within each probe. Concentration data were normally distributed after log-transformation, and all analyses were performed on log-transformed data. Within each treatment, samples were compared to baseline by one-way ANOVA followed by Dunnett's test, with $P < 0.05$ considered significant.

Results

Cerebrospinal Fluid Sampling in Sheep

The groups did not differ in baseline CSF concentrations of neurotransmitters (table 1). Morphine, 1 mg/kg, but not saline, caused an increase in CSF concentrations of norepinephrine (fig. 1). Peak CSF concentrations of norepinephrine occurred at a median of 15 min (25th to 75th percentiles = 10–22.5 min) after morphine injection. Although 0.5 mg/kg morphine numerically increased CSF norepinephrine concentrations, values failed to differ from baseline at any time by Kruskal-Wallis test, and peak concentrations did not differ from baseline (fig. 2). Power analysis revealed a difference of this magnitude would have required study of roughly twice as many animals (22) to demonstrate statistical significance. There was a dose-dependent effect of intravenous morphine on CSF norepinephrine,

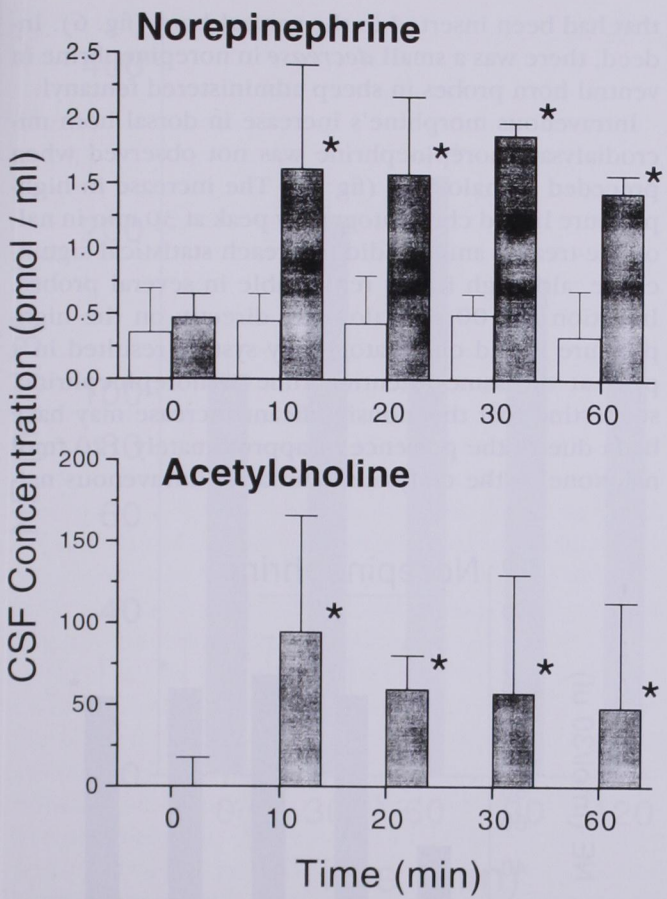

Fig. 1. Effect of saline (open bars) or morphine, 1.0 mg/kg intravenous (gray bars) on cerebrospinal fluid concentrations of norepinephrine (upper panel) and acetylcholine (lower panel). Each value represents the median + 75th percentiles of 5–10 animals. *$P < 0.05$ *versus* time 0.

with 1 mg/kg, but not 0.5 mg/kg significantly increasing CSF norepinephrine (fig. 2).

In antagonist experiments, both intrathecal idazoxan and intravenous naloxone antagonized the effect of morphine, 1 mg/kg on CSF norepinephrine concentration, as individual time points and peak concentrations failed to differ from baseline (fig. 2).

Acetylcholine was undetectable (<10 pmol/ml) in all but three samples before and after saline injection. In contrast to saline, 1 mg/kg morphine caused an increase in CSF concentrations of acetylcholine (fig. 1; lower panel). Peak CSF concentrations of acetylcholine occurred at a median of 10 min (25th to 75th percentiles = 10–20 min) after morphine injection. Morphine (0.5 mg/kg) increased CSF acetylcholine concentrations significantly 10 and 60 min after injection, and peak acetylcholine concentrations were significantly higher than baseline in this group (fig. 2). In antagonist experiments, both intrathecal idazoxan and intravenous naloxone antagonized the effect of morphine on CSF acetylcholine concentration, as individual time points and peak concentrations failed to differ from baseline (fig. 2).

In contrast to morphine's effect on CSF norepinephrine and acetylcholine, there was no effect on CSF epinephrine or dopamine after either dose of morphine (fig. 3).

Spinal Cord Microdialysis in Sheep

Surgery, including implantation of microdialysis probes, was completed within 3 h in all cases. Arterial blood gas tensions and pH levels were stable throughout the experiments (data not shown). Postmortem ex-

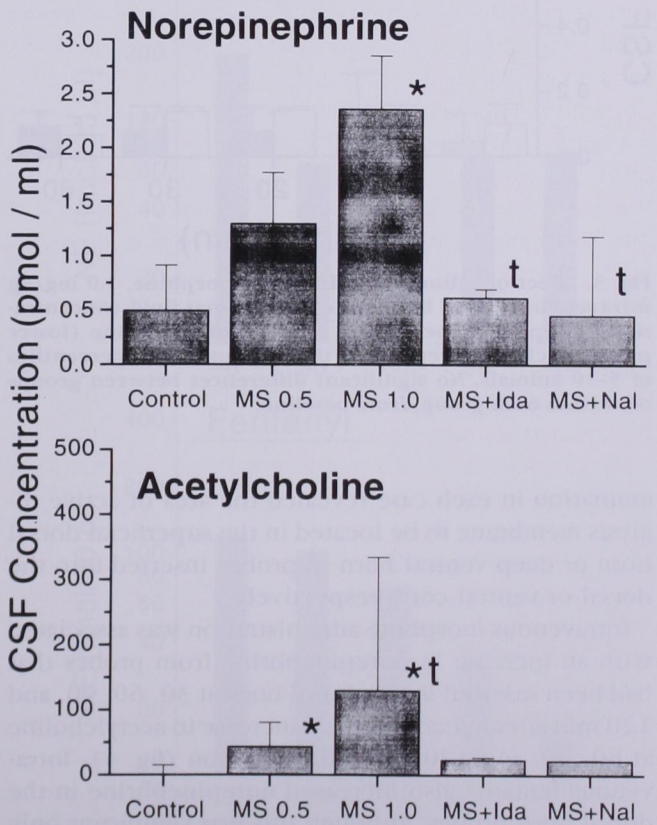

Fig. 2. Peak cerebrospinal fluid concentrations of norepinephrine (upper panel) and acetylcholine (lower panel) after intravenous injection of morphine (MS) alone (0.5 and 1.0 mg/kg), morphine plus intrathecal idazoxan (MS + Ida) or morphine plus intravenous naloxone (MS + Nal). Each value represents the median + 75th percentiles of five to ten animals. *$P < 0.05$ *versus* value before intravenous injection. †$P < 0.05$ *versus* intravenous 1.0 mg/kg morphine.

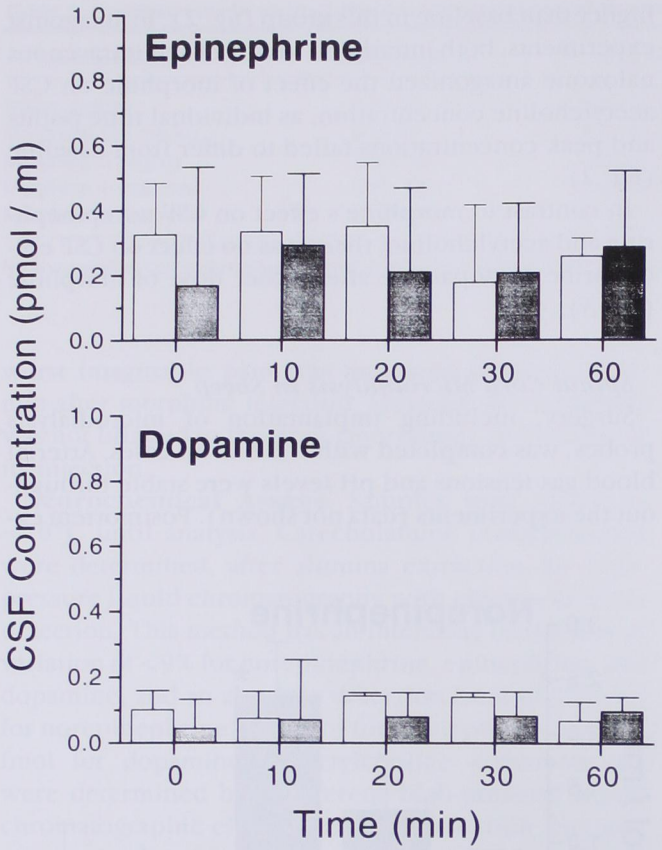

Fig. 3. Effect of saline (open bars) or morphine, 1.0 mg/kg intravenous (closed bars) on cerebrospinal fluid concentrations of epinephrine (upper panel) and dopamine (lower panel). Each value represents the median + 75th percentiles of 5–10 animals. No significant differences between groups or, within each group, from baseline.

amination in each case revealed the area of active dialysis membrane to be located in the superficial dorsal horn or deep ventral horn in probes inserted into the dorsal or ventral cord, respectively.

Intravenous morphine administration was associated with an increase in norepinephrine from probes that had been inserted in the dorsal horn at 30, 60, 90, and 120 min after injection and an increase in acetylcholine at 60, 90, and 120 min after injection (fig. 4). Intravenous fentanyl also increased norepinephrine in the dorsal horn probes, although this was significant only at 60 min after fentanyl injection (fig. 5). We note that baseline concentrations of norepinephrine varied numerically among various treatments, likely reflecting the correlation among probes in a given animal but variability among animals. Neither morphine nor fentanyl, however, increased norepinephrine from probes that had been inserted in the ventral horn (fig. 6). Indeed, there was a small *decrease* in norepinephrine in ventral horn probes in sheep administered fentanyl.

Intravenous morphine's increase in dorsal horn microdialysate norepinephrine was not observed when preceded by naloxone (fig. 7). The increase in high-pressure liquid chromatography peak at 30 min in naloxone-treated animals did not reach statistical significance, although it was remarkable in several probes. Injection of 100 pg naloxone, directly on the high-pressure liquid chromatography system resulted in a peak at the same retention time as norepinephrine, suggesting that this nonsignificant increase may have been due to the presence of approximately 120 fmol naloxone in the dialysate. Similarly, intravenous nal-

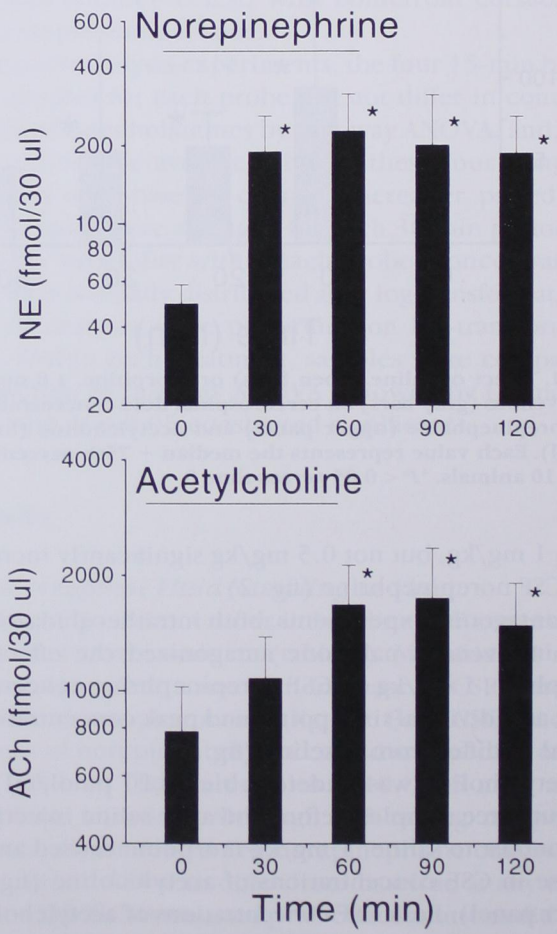

Fig. 4. Effect of 1 mg/kg intravenous morphine on microdialysate concentrations of norepinephrine (upper panel) or acetylcholine (lower panel) from probes inserted in dorsal horn of the spinal cord. Each bar represents the mean ± SEM of 5–8 probes in three animals. Morphine was injected at time 0. *$P < 0.05$ *versus* baseline (time 0).

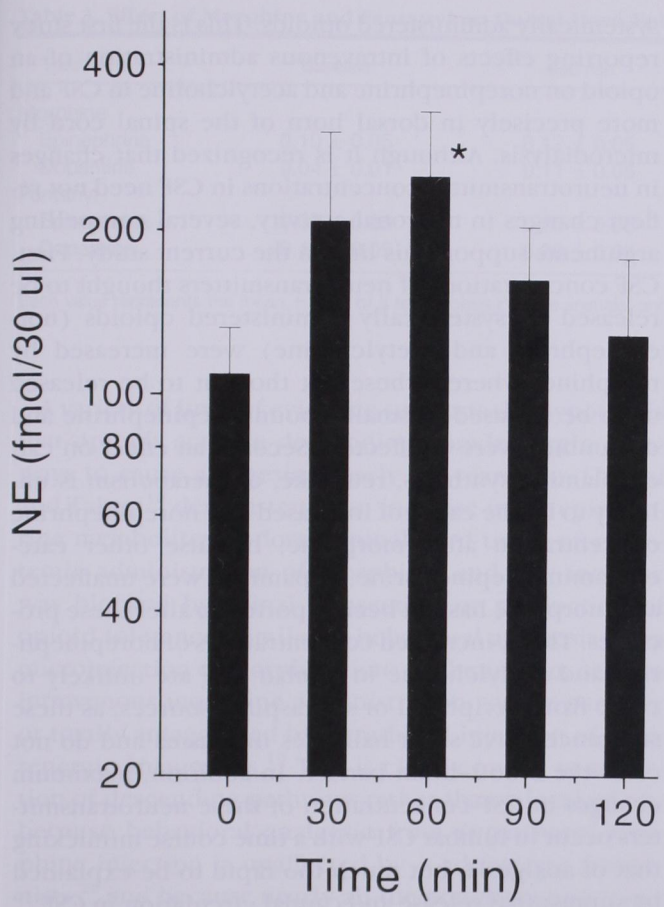

Fig. 5. Effect of 10 μg/kg intravenous fentanyl on microdialysate concentrations of norepinephrine from probes inserted in dorsal horn of the spinal cord. Each bar represents the mean ± SEM of five probes in three animals. Fentanyl was injected at time 0. *$P < 0.05$ *versus* baseline (time 0).

oxone pretreatment in fentanyl-treated animals resulted in a nonsignificant increase in dorsal horn microdialysate norepinephrine 30 min after injection, and blocked the increase seen in animals receiving fentanyl alone (table 2).

In contrast to their effects on norepinephrine, neither opioid increased epinephrine or dopamine microdialysate concentrations in probes inserted into the dorsal horn (table 3). Epinephrine and dopamine also were not increased after opioid treatment in probes inserted into the ventral horn (data not shown). Finally, these monoamines from dorsal horn probes were unaffected by opioid + naloxone treatment or by opioid treatment after cervical spinal cord transection (data not shown).

Cerebrospinal Fluid Sampling in Human

Spinal neostigmine, injected 24 h before morphine as part of another study,[14] had increased CSF acetylcholine, but both CSF acetylcholine and CSF norepinephrine had returned to values similar to those before neostigmine injection at the time of morphine injection. Intravenous morphine increased CSF norepinephrine and acetylcholine, with a time course similar to that of actions on end-tidal CO_2 (fig. 8). Visual analog scale pain report to noxious cold stimulation decreased from 8.8 cm before morphine to 5.4 cm 30 min after morphine administration in this nonblinded volunteer.

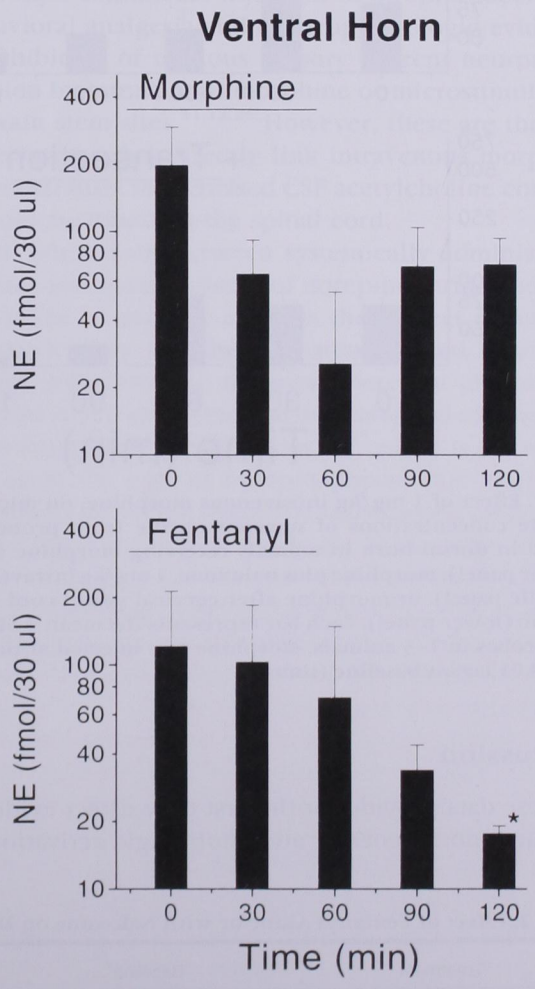

Fig. 6. Effect of 1 mg/kg intravenous morphine (upper panel) or 10 μg/kg intravenous fentanyl, on microdialysate concentrations of norepinephrine from probes inserted in ventral horn of the spinal cord. Each bar represents the mean ± SEM of 3–6 probes in three animals. Morphine or fentanyl was injected at time 0. *$P < 0.05$ *versus* baseline (time 0).

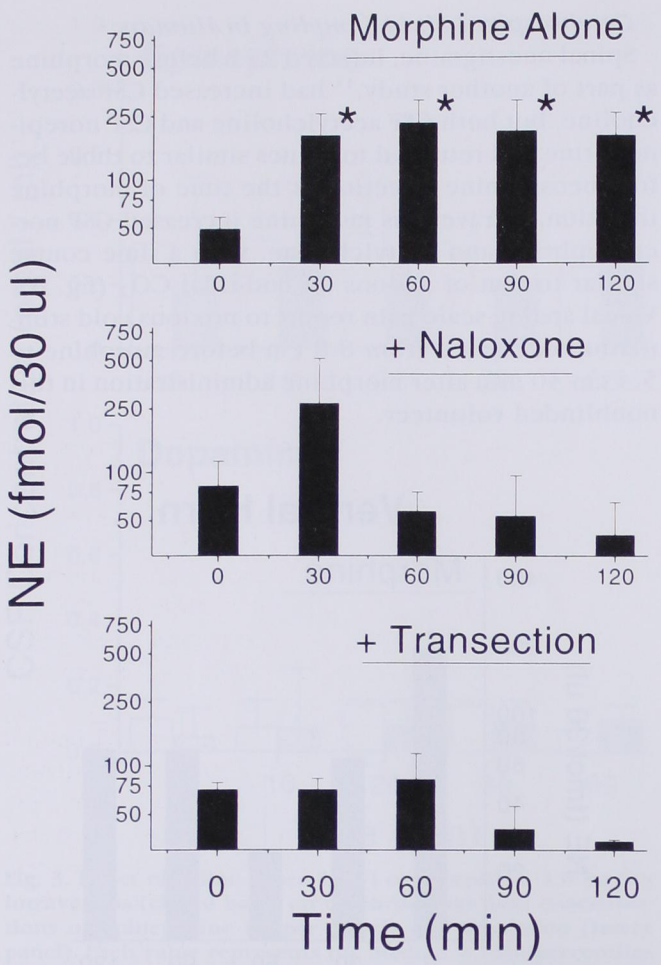

Fig. 7. Effect of 1 mg/kg intravenous morphine, on microdialysate concentrations of norepinephrine from probes inserted in dorsal horn in animals receiving morphine alone (upper panel), morphine plus naloxone, 1 mg/kg intravenous (middle panel), or morphine after cervical spinal cord transection (lower panel). Each bar represents the mean ± SEM of 3–8 probes in 1–3 animals. Morphine was injected at time 0. *$P < 0.05$ *versus* baseline (time 0).

Discussion

These data provide for the first time direct evidence of spinal noradrenergic and cholinergic activation by systemically administered opioids. This is the first study reporting effects of intravenous administration of an opioid on norepinephrine and acetylcholine in CSF and more precisely in dorsal horn of the spinal cord by microdialysis. Although it is recognized that changes in neurotransmitter concentrations in CSF need not reflect changes in neuronal activity, several compelling arguments support this link in the current study. First, CSF concentrations of neurotransmitters thought to be released by systemically administered opioids (norepinephrine and acetylcholine) were increased by morphine, whereas those not thought to be released or to be released in small amounts (epinephrine and dopamine) were unaffected. Second, an effect on catecholamine synthesis, reuptake, or metabolism is unlikely to be the cause of increased CSF norepinephrine concentration after morphine, because other catecholamines (epinephrine, dopamine) were unaffected and morphine has not been reported to alter these processes. Third, increased concentrations of norepinephrine and acetylcholine in lumbar CSF are unlikely to come from peripheral or supraspinal sources, as these substances have short half-lives in plasma and do not cross the blood–brain barrier. In addition, maximum changes in CSF concentration of these neurotransmitters occur in lumbar CSF with a time course mimicking that of analgesia, but much too rapid to be explained by supraspinal release and caudal circulation in CSF.[16] Fourth, the doses of morphine used in these experiments have been shown to produce behavioral analgesia to a noxious thermal stimulus in sheep (A. Waterman, personal communication and reference 17) and are considered clinically effective in humans. Finally, microdialysis results mirrored changes in concentrations of neurotransmitters in CSF. As such, these acute changes in CSF norepinephrine and acetylcholine concentrations after intravenous morphine are likely to reflect local spinal neurotransmitter release, and these observations provide unique evidence for the relevance of these neurotransmitters to the action of systemically administered opioids.

Table 2. Effect of Fentanyl Alone or with Naloxone on Dorsal Horn Microdialysate Concentrations of Norepinephrine

Treatment	Baseline	+30 min	+60 min	+90 min	+120 min
Intravenous fentanyl	0.11 ± 0.02	0.21 ± 0.09	0.25 ± 0.08*	0.16 ± 0.04	0.13 ± 0.05
Intravenous fentanyl + naloxone	0.05 ± 0.01	0.24 ± 0.18	0.14 ± 0.13	0.035 ± 0.006	0.031 ± 0.013

Each value represents the mean ± SEM of 3 to 8 probes in three animals, and is expressed as pmol/30 μl.

* $P < 0.05$ *versus* baseline.

Table 3. Effect of Morphine and Fentanyl on Dorsal Horn Epinephrine and Dopamine Microdialysate Concentrations

Treatment	Baseline	+30 min	+60 min	+90 min	+120 min
Morphine					
Epinephrine	0.10 ± 0.05	0.12 ± 0.05	0.11 ± 0.06	0.08 ± 0.03	0.10 ± 0.03
Dopamine	0.04 ± 0.01	0.11 ± 0.06	0.08 ± 0.03	0.05 ± 0.02	0.04 ± 0.01
Fentanyl					
Epinephrine	0.33 ± 0.25	0.20 ± 0.20	0.09 ± 0.04	0.08 ± 0.05	0.08 ± 0.04
Dopamine	0.19 ± 0.09	0.20 ± 0.09	0.22 ± 0.10	0.17 ± 0.07	0.20 ± 0.12

Each value represents the mean ± SEM of 3 to 8 probes in three animals, and is expressed as pmol/30 μl. There were no significant differences.

A variety of lines of evidence supports the hypothesis that opioids activate descending noradrenergic pathways to cause analgesia. Nearly 20 years ago, Shiomi and Takagi[18] demonstrated an increase in norepinephrine metabolites in dorsal spinal cord tissue after systemic administration of morphine, and this increase was blocked by spinal transection or production of opioid tolerance. Similarly, behavioral analgesia from microinjection of morphine into the brain stem or from intravenous morphine administration can be partially or totally antagonized by intrathecal injection of α adrenergic antagonists.[19] This is clearly owing to activation of descending pathways rather than a local effect, because behavioral analgesia from *intrathecal* morphine injection is unaffected by α adrenergic antagonists,[20] and because nearly all the noradrenergic input to the spinal cord comes from neurons with cell bodies in the brain stem, not the spinal cord.[21,22]

The above anatomic and functional studies support the hypothesis of opioid-induced descending noradrenergic inhibition, but do not directly test it by measurement of norepinephrine release. The effect of morphine on CSF[23] or extracellular concentrations of serotonin in the spinal cord dorsal horn, as measured by microdialysis,[24] have been performed, but not the effect on norepinephrine. A preliminary report demonstrates increased CSF concentrations of norepinephrine in awake humans after intravenous morphine,‖ supporting the relevance of these observations in animals to clinical practice. However, this is the first report to examine the pharmacology of intravenous morphine-induced changes in CSF norepinephrine concentrations in the nonanesthetized state.

There are also several lines of evidence that opioids produce analgesia in part by activation of cholinergic pathways. Intrathecal injection of atropine decreases behavioral analgesia and electrophysiologic evidence of inhibition of noxious sensory afferent neurotransmission by intravenous morphine or microstimulation of brain stem sites.[11,12,25] However, these are the first observations that directly link intravenous morphine administration to increased CSF acetylcholine concentrations surrounding the spinal cord.

The interaction between systemically administered opioids and spinal release of norepinephrine and acetylcholine is unclear, and was the subject of our experiment with intrathecally administered idazoxan. Unlike noradrenergic neurons, there are cholinergic neurons in the spinal cord as well as spinal cholinergic fibers from brain stem neurons,[26,27] and it is not as certain as in the case of norepinephrine that morphine increases CSF acetylcholine by activation of brain stem cholinergic neurons in the brain stem. Some experiments in rodents and sheep demonstrate an increase in CSF acetylcholine after intrathecal injection of α_2-adrenergic agonists and an enhancement of analgesia from intrathecal α_2-adrenergic agonists by cholinesterase inhibitors.[13,28,29] These results suggest that spinally released norepinephrine may act on α_2-adrenoceptors on spinal cholinergic neurons to cause acetylcholine release and analgesia. Based on these results, we hypothesized that CSF norepinephrine concentrations should increase by similar amounts after intravenous morphine alone or morphine plus intrathecal idazoxan, but that morphine-induced increases in acetylcholine would be blocked by idazoxan. Unexpectedly, idazoxan blocked morphine-induced increases in both CSF norepinephrine and acetylcholine concentrations, so we were unable to adequately test the proposed α_2-adrenoceptor link between these two. The mechanism by which idazoxan blocked morphine-induced increases in CSF

‖ Kimura S, Arai T: The effects of pain and systemically administered opioids on the concentration of noradrenaline (NA) and 5-hydroxyindole acetic acid (5-HIAA) in human cerebrospinal fluid. Proceedings of the 7th World Congress on Pain, 1993 (abstract).

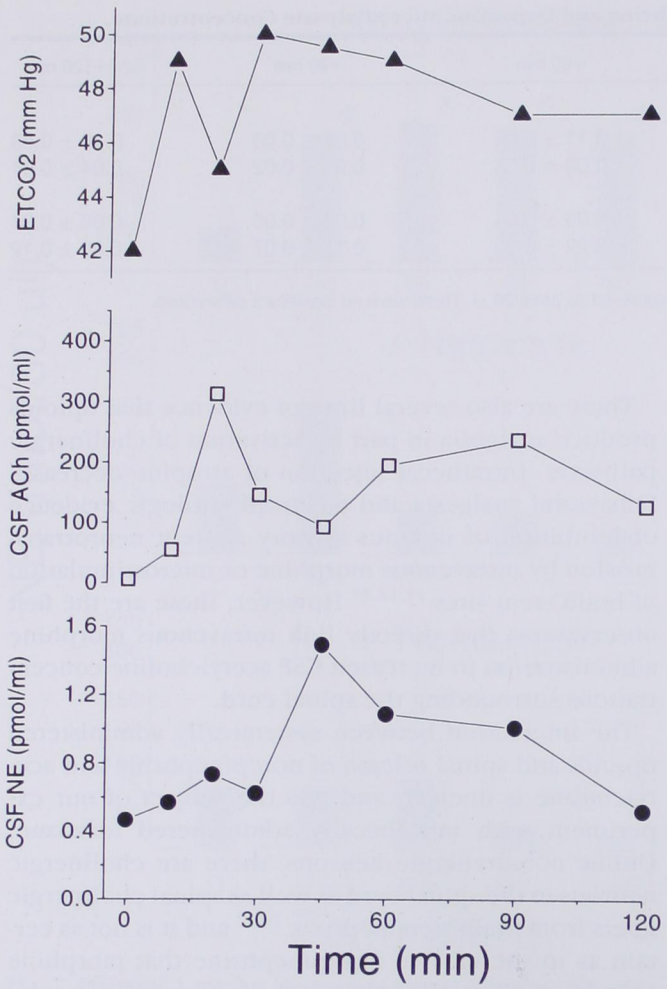

Fig. 8. Effect of 10 mg intravenous morphine on lumbar cerebrospinal fluid concentrations of norepinephrine (●) and acetylcholine (□) in a single human volunteer. In the upper panel are simultaneous determinations of end-tidal carbon dioxide (ET_{CO_2}; ▲).

norepinephrine concentrations is unclear, as one would expect blockade or presynaptic α_2-adrenoceptors by this agent, if anything, to increase CSF norepinephrine.

Microdialysis experiments confirm and validate the CSF sampling experiments because qualitatively similar results were obtained with either CSF or dorsal horn interstitial fluid sampling of acetylcholine and monoamines. Lack of increase of norepinephrine in ventral horn microdialysate samples supports a specific release of this neurotransmitter in the dorsal horn. Increases in norepinephrine concentrations after intravenous injection of two structurally dissimilar opioids and blockade of these increases by intravenous naloxone supports an opioid mechanism rather than a nonspecific action of morphine. Finally, blockade of morphine-induced increase in norepinephrine by cervical spinal cord transection supports the hypothesis that bulbospinal pathways were activated by morphine to cause this effect.

Similar increases in CSF norepinephrine and acetylcholine after a clinically effective dose of intravenous morphine were observed in the human as in the sheep experiments. Although pain scores could be considered suspect in this nonblinded volunteer, there is little doubt that 10 mg intravenous morphine produces some analgesia in postoperative patients. These observations in a single individual, while not definitive, are consistent with the animal results and suggest this line of investigation may be fruitful in drug development to enhance opioid action. Spinal administration of norepinephrine reuptake inhibitors enhance morphine analgesia in animals.[30] Although absence of preclinical safety assessment precludes spinal injection of these agents in humans, systemic administration enhances morphine analgesia,[31,32] and development of such agents for spinal administration may lead to even greater potentiation. Similarly, systemic administration of either physostigmine[33,34] or metoclopramide,[35,36] which inhibit breakdown of acetylcholine, causes analgesia itself and enhances analgesia from systemically administered opioids. Preclinical safety assessment of the cholinesterase inhibitor, neostigmine, for spinal administration is completed,[37,38] and clinical trials will test the interaction between spinal neostigmine and systemic opioids.

In summary, intravenous morphine, in a dose that produces antinociception, increases lumbar CSF concentrations of norepinephrine and acetylcholine in conscious sheep in a naloxone-reversible manner. Microdialysis experiments suggest these increases in norepinephrine and acetylcholine in CSF reflect local release of these neurotransmitters from the spinal cord dorsal horn as a result of bulbospinal pathway activation. These data support opioid-induced activation of descending noradrenergic pathways in sheep and humans, although whether increased spinal cholinergic activity after morphine is owing to activation of descending pathways or norepinephrine-induced activation of spinal cholinergic interneurons is not clear.

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