Enhancement of Analgesia from Systemic Opioid in Humans by Spinal Cholinesterase Inhibition

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ABSTRACT

Intravenous opioids cause analgesia and increase release of ACh in spinal cord dorsal horn in animals, and these effects are enhanced by intrathecal neostigmine injection. The purpose of the current study was to test whether intrathecal neostigmine enhanced analgesia and increased cerebrospinal fluid concentrations of ACh over those induced by i.v. alfentanil in volunteers, and also to test whether neostigmine enhanced alfentanil-induced side effects. After animals studies committee approval, 40 healthy volunteers received an intrathecal injection of saline or neostigmine (50, 100 or 200 μg) followed in 60 min by a computer-controlled, stepped i.v. infusion of alfentanil to escalating targeted plasma concentrations. Pain report to hand and foot immersion in ice water, sedation, nausea, weakness, vital signs, end-tidal CO₂ and oxyhemoglobin saturation were measured 60 min after spinal injection and at the end of each 20-min alfentanil infusion. Cerebrospinal fluid was sampled once after drug administration. Intrathecal neostigmine alone caused analgesia in the foot but not in the hand, and was accompanied by leg weakness, whereas IV alfentanil alone caused equivalent analgesia in both the hand and the foot and was accompanied by nausea, sedation, increased end-tidal CO₂ and decreased oxyhemoglobin saturation. Neostigmine enhanced analgesia but not respiratory effects induced by i.v. alfentanil; it also enhanced nausea and sedation. Intravenous alfentanil increased cerebrospinal fluid ACh concentration, and neostigmine enhanced this change. These data in humans are consistent with a spinal cholinergic mechanism of i.v. opioid analgesia. Because neostigmine enhances both analgesia and side effects induced by i.v. alfentanil, the clinical utility of their use in combination will depend on the relative strength of these interactions.

Systemically administered opioids produce analgesia by actions in the periphery, the brain and the spinal cord. Whereas these agents selectively activate opioid receptors, the end results of opioid receptor activation may ultimately be mediated through other receptor types. For example, opioid administration, either into brainstem loci such as the periaqueductal grey (Yaksh and Tyce, 1979) or dorsal raphe (Tseng and Tang, 1989) or systemically (Bouaziz et al., 1996) produces behavioral analgesia by activation of descending inhibitory mechanisms that involve the release of monamines, especially norepinephrine. Anatomically, this effect is correlated with noradrenergic innervation of the cord from midbrain and medullary loci, which are directly or indirectly stimulated by opioids (Kwiat and Basbaum, 1992). This spinal release of norepinephrine diminishes substance P release from primary Aδ and C afferents (Kuraishi et al., 1985) and reduces the response of the dorsal horn neurons to noxious stimulation (Headley et al., 1978). There is also spinal cholinergic stimulation that is associated with systemic opioid administration (Chiang and Zhuo, 1989). The cholinergic relationship to the descending noradrenergic system is unclear. There are cholinergic neurons in the spinal cord in addition to cholinergic fibers that descend from the brainstem (Jones et al., 1986; Barber et al., 1984). There is also a high density of both alpha-2 adrenergic and cholinergic ligand binding in Rexed's lamina I and II (Gillberg et al., 1988; Seybold and Elde, 1984; Unnerstall et al., 1984). Opioid-stimulated release of norepinephrine activates spinal cholinergic neurons (Detweiler et al., 1993), and spinally released norepinephrine and ACh have been hypothesized to be partially responsible for analgesia induced by i.v. opioids (Chiang and Zhuo, 1989).

After preclinical toxicity screening (Hood et al., 1995a; Yaksh et al., 1995), the cholinesterase inhibitor neostigmine was recently introduced into clinical trials. In healthy volunteers, lumbar intrathecal neostigmine administration increases ACh concentrations in CSF and produces analgesia to noxious cold stimulation, which is greater in the foot than in the hand (Hood et al., 1995b). These results are consistent with tonic spinal release of ACh in normal humans, the actions of which are enhanced by neostigmine, yielding an-

ABBREVIATIONS: CSF, cerebrospinal fluid; EC₅₀, effective concentration producing a 50% maximum response; HPLC, high-pressure liquid chromatography; % MPE, percent maximum possible effect; SpO₂, oxyhemoglobin saturation; VAS, visual analog scale.
algesia by a spinal mechanism. The purpose of the current study was to determine whether i.v. alfentanil produced analgesia and increased CSF concentrations of ACh and whether these effects could be enhanced by intrathecal neostigmine injection.

The most worrisome side effect of i.v. opioids is respiratory depression, although nausea and sedation from opioids limit their utility in many patients. Intrathecal neostigmine does not cause respiratory depression in volunteers, but it does produce dose-dependent nausea, leg weakness and sedation (Hood et al., 1995b). A secondary purpose of the current study was to determine whether these drugs might also enhance each others' side effects.

**Methods**

After human studies committee approval and written informed consent, 40 healthy American Society of Anesthesiologists physical status 1 or 2 volunteers reported to the General Clinical Research Center at 7:00 A.M., having had nothing to eat or drink since midnight. Women of child-bearing potential had a negative urine pregnancy test before enrollment. An i.v. catheter was inserted into one arm for drug administration and into the other arm for blood sampling.

To determine analgesia, volunteers immersed a hand and, 5 min later, a foot (random order) in stirred ice water for 60 sec and rated their pain using a pain magnitude estimate. The pain magnitude estimate is defined as a verbal numerical pain scale where the volunteer selects the numerical value that corresponds to the pain experienced during the base-line pain assessment. There are no restrictions or suggestions as to the appropriate initial pain magnitude score (value). The volunteer was instructed to remember the base-line pain and her or his assigned value and, upon subsequent pain assessment, to assign a numerical value relative to the base-line pain. That is, if a subsequent pain assessment is half the base-line pain, then the pain magnitude score should be half the base-line score. This pain magnitude score under these conditions has been demonstrated to be a linear response to an escalating pain stimulus (LaMotte et al., 1983). Volunteers also completed a 10-cmVAS for nausea (anchors: no nausea, as nauseated as possible), sedation (anchors: wide awake, as sleepy as possible) and weakness (anchors: no weakness, as weak as possible). Blood pressure and HR were determined using a noninvasive oscillometric device. SpO₂ and end-tidal CO₂ were determined with a pulse oximeter and end-tidal gas analyzer, respectively.

After base-line measures were obtained, volunteers received a lumbar intrathecal injection while in the lateral decubitus position and then were positioned supine with the head of the bed elevated 30° to 45° for the remainder of the study. A research nurse blind to drug treatment measured and recorded outcome parameters. In accordance with a double-blind, computer-generated, randomized design, volunteers received an intrathecal injection of 2 ml 5% dextrose via a #27 Whitacre needle inserted at a lumbar interspace, via a #27 Whitacre needle inserted at a lumbar interspace, the hood et al., 1995b). The 50-μg dose produced minimal analgesia in that study, and the 200-μg dose was the maximum dose we chose to administer because of the increasing incidence of nausea in volunteers from intrathecal neostigmine. Experimental measures were repeated 60 min later, and measurements at this time were considered to reflect the effects of neostigmine alone.

When the measures taken 60 min after intrathecal injection had been obtained, a computer-controlled i.v. infusion, using the STAN-PUMP (Shafer et al., 1990) algorithm and the Shafer body surface area-adjusted kinetic subset, was begun, to the initial targeted plasma alfentanil concentration. Venous blood was sampled for alfentanil assay 20 min later, and the infusion was increased to the intermediate targeted concentration. Venous blood was sampled for alfentanil and norepinephrine assay after another 20 min, and the infusion was increased to the largest targeted concentration. Another 20 min later, venous blood was sampled for alfentanil and norepinephrine assay, and the infusion was discontinued. Volunteers were allowed to leave 2 hrs later in the care of another adult. The first three volunteers received alfentanil targeted to 10, 30 and 100 ng/ml, and thereafter the protocol was amended to alter the targeted alfentanil concentrations depending on the composition of the intrathecal injection. For subsequent volunteers, alfentanil targeted concentrations were 50, 100 and 200 ng/ml if the volunteer was randomized to the intrathecal saline group and were 25, 50 and 100 ng/ml if the volunteer was randomized to the intrathecal neostigmine group. Volunteers received less alfentanil if randomized to receive intrathecal neostigmine and more alfentanil if receiving intrathecal saline. Because we were anticipating an enhancement of analgesia from alfentanil by neostigmine, we selected these drug combinations to have all dose-response curves cover roughly the same range of efficacy.

Experimental measures were obtained before and 60 min after intrathecal injection and then at the end of each 20-min i.v. infusion before the plasma target was changed or the infusion discontinued. This study design allowed assessment of three study groups using two groups of volunteers: intrathecal saline and alfentanil infusion (alfentanil alone group), intrathecal neostigmine with outcome parameters assessed 60 min after intrathecal drug injection (neostigmine alone group) and 60 min after intrathecal neostigmine, a stepped alfentanil infusion (intrathecal neostigmine and i.v. alfentanil to stepped, targeted plasma concentrations).

CSF (1 ml) was sampled through the spinal needle, before intrathecal injection, for ACh and norepinephrine analysis. CSF was sampled a second time at the end of the largest targeted alfentanil infusion, via a #27 Whitacre needle inserted at a lumbar interspace, and analyzed for ACh, norepinephrine and alfentanil.

Episodes of emesis were noted. Severe nausea or vomiting was treated with droperidol, 0.5 mg i.v., repeated once in 5 min, and then with ondansetron, 4 mg i.v., repeated once in 5 to 15 min, or phenergan, 12.5 mg i.v., repeated once in 5 to 15 min, or naloxone, 100 μg, repeated once in 5 to 15 min. Drug treatment was prescribed for increases or decreases in blood pressure or HR of >25% or if symptomatic, but no volunteer met criteria for treatment. Supplemental oxygen was administered via nasal cannulas for SpO₂ <90%. Oxygen was withdrawn for 5 min at the time of experimental measures and then was reinstated if <90%.

ACh was analyzed by HPLC with electrochemical detection, with interassay coefficient of variation of 98% and limit of detection of 50 fmol (Detweiler et al., 1992). Norepinephrine was analyzed by HPLC with electrochemical detection, with interassay coefficient of variation of <9% and limit of detection of 12 fmol (Eisenach et al., 1992).

ACh and alfentanil was analyzed by radioimmunoassay, with interassay coefficient of variation of 4% and detection limit of 0.05 ng/ml (Bjorkman et al., 1989).

Unless otherwise stated, data are presented as mean ± S.E.M. Pain magnitude estimates were converted to % MPE by dividing the difference between the pain report before drug to that after drug by the pain report before drug, multiplied by 100. This is analogous to a standard MPE calculation, where the cutoff value is complete pain relief (pain magnitude estimate of zero). The effects of neostigmine, alfentanil plasma concentration and their possible interaction with respect to sedation, nausea, weakness, blood pressure, HR, SpO₂, respiratory rate, end-tidal CO₂ and % MPE in foot and hand were analyzed using the Proc Mixed protocol in SAS (SAS Institute, Cary, NC). The experimental design consisted of a mixed-effects model with volunteer as the random factor and repeated factors within each volunteer for the various neostigmine and alfentanil drug treatments over time.
The effects of neostigmine and alfentanil concentrations on the outcome parameters were tested both as linear and as quadratic effects within the analysis of covariance model (mixed-effects model). The quadratic terms allowed for curvilinear effects to be modeled, but the linear-effect model best fit the data. Analysis for additivity or nonadditivity was based on the assumption of linear dose effects for each drug alone. A significant interaction for neostigmine and alfentanil alone, in this mixed-effects model, would indicate nonadditivity, and a nonsignificant P value would imply additivity. Covariates for each volunteer's gender, age, height and weight were included in the analyses.

The (EC50) value of alfentanil for % MPE to hand and foot testing was calculated at each neostigmine dose. First, linear regressions of % MPE to hand and foot testing over alfentanil concentrations were performed for each neostigmine concentration. The concentrations of alfentanil needed to achieve the EC50 values were then determined directly from the regression equations. The standard errors of the EC50 estimates were determined from the regression coefficient standard errors using Taylor series approximations (Casella and Berger, 1990). Analysis of variance (ANOVA) was used to test for differences in EC50 values of alfentanil at the various neostigmine doses. Contrasts were included in the statistical model whenever individual paired or complex comparisons were desired. Adjustments were made for multiple comparisons using Fisher’s protected LSD when appropriate. P < .05 was considered significant.

Results

Volunteers were 31 ± 1.1 years old, 172 ± 2 cm in height and 79 ± 2.5 kg in weight. There were 17 males and 23 females. All volunteers completed the study protocol, and there were no side effects beyond the day of the experiment, except headache after dural puncture, which resolved spontaneously in two volunteers and resolved with epidural blood-patch therapy in one volunteer. Two of five male volunteers receiving the 200-µg dose of intrathecal neostigmine experienced ejaculation during the alfentanil infusion. Plasma alfentanil concentrations were approximately 20% less than those targeted: 22 ± 1.2 ng/ml (range 11–45 ng/ml) for the 25-ng/ml target, 44 ± 2.2 ng/ml (range 17–87 ng/ml) for the 50-ng/ml target, 78 ± 3.2 ng/ml (range 25–132 ng/ml) for the 100-ng/ml target and 166 ± 12 ng/ml (range 132–228 ng/ml) for the 200-ng/ml target.

Analgesia. Intrathecal neostigmine injection produced dose-dependent analgesia to noxious cold stimulation in the foot, but not in the hand. At each neostigmine dose, analgesia was greater in the foot than in the hand (fig. 1). In contrast, i.v. alfentanil produced analgesia that correlated with alfentanil plasma concentration and was equivalent in foot and hand (fig. 1). The dose-response curves for alfentanil alone and neostigmine alone appear linear. Statistical analysis confirms this; use of the quadratic model did not significantly improve the dose-response curve fit for either drug alone. Thus linear dose-response curves were used at our drug dose levels, and interaction terms in subsequent statistical analyses were deemed sufficient indications of drug interaction.

Alfentanil-induced analgesia was enhanced in both foot and hand by neostigmine (fig. 2). Statistical analysis revealed this interaction to be mathematically additive in the foot (P = .10), but synergistic in the hand (P < .005). This can be appreciated visually in figure 2 by comparing the parallel shift in alfentanil concentration-analgesia response in the foot in the presence of increasing doses of neostigmine with the increasing slope of this response in the hand. However, because of the minimal effect of neostigmine alone in the hand, the absolute magnitude in shift of alfentanil dose-response induced by neostigmine was greater in the foot than in the hand. As demonstrated in figure 3, the EC50 value of alfentanil was reduced by intrathecal neostigmine to noxious stimulation to a greater extent in the foot than in the hand (P < .05).

Fig. 1. Analgesia, expressed as % MPE in the foot (□) and the hand (●) observed 60 min after intrathecal neostigmine alone (left panel) and as a function of plasma alfentanil concentrations after i.v. alfentanil alone or in the absence of intrathecal neostigmine (right panel). * P < .05 vs. predrug values; † P < .05 vs. hand.

Fig. 2. Analgesia, expressed as % MPE in the foot (left panel) and the hand (right panel) observed as a function of plasma alfentanil concentrations after i.v. alfentanil in the absence of intrathecal neostigmine (●) or in the presence of neostigmine 50 µg (▲), 100 µg (▲) or 200 µg (▼). Neostigmine enhanced alfentanil-induced analgesia in an additive manner in the foot, an effect indicated graphically by the parallel concentration-effect curves, and in a synergistic manner in the hand, indicated by divergent concentration-effect curves.

Fig. 3. EC50 value for alfentanil in the foot (□) and the hand (●) in the absence or presence of intrathecal neostigmine, 50, 100 or 200 µg. * P < .05 vs. i.v. alfentanil without intrathecal neostigmine.
Side effects. As with the analgesia analysis, the linear response model best fit the data describing side effects. Neostigmine had no effect on respiration, as determined by SpO₂ and end-tidal CO₂. In contrast, alfentanil produced plasma concentration-dependent decreases in SpO₂ (P < 0.001) and increases in end-tidal CO₂ (P < 10⁻⁵; fig. 4). Three volunteers receiving alfentanil plus intrathecal saline and three receiving alfentanil plus intrathecal neostigmine required supplemental oxygen following the largest plasma targeted alfentanil infusion. One of the volunteers receiving alfentanil plus intrathecal saline received naloxone, 100 µg, to treat SpO₂ of <90% despite supplemental oxygen. Neostigmine had no effect on alfentanil-induced changes in SpO₂ or end-tidal CO₂ (fig. 4).

Alfentanil, but not neostigmine, produced dose-dependent sedation (P < 10⁻⁵; fig. 5). Neostigmine increased alfentanil-induced sedation in a synergistic manner (P = 0.017). In contrast, neostigmine, but not alfentanil, produced dose-dependent subjective weakness (P < 0.005; fig. 5). Alfentanil increased neostigmine-induced weakness in a synergistic manner (P < 10⁻⁴).

Alfentanil alone, but not neostigmine, tended to cause nausea (P = 0.07), although of small magnitude (fig. 6). Neostigmine synergistically increased nausea from alfentanil (P < 0.05). No volunteer received more than one treatment for severe nausea or vomiting in the alfentanil plus intrathecal saline group, whereas 2, 3 and 3 volunteers received more than one such treatment in the alfentanil plus intrathecal neostigmine groups (50, 100 and 200 µg, respectively). Similarly, 2 volunteers vomited during alfentanil infusion in the intrathecal saline group, but 6, 5 and 6 volunteers vomited in the intrathecal neostigmine groups (50, 100 and 200 µg, respectively).

Alfentanil alone produced a small (<10%) but statistically significant reduction in HR (P = 0.02). Neostigmine alone did not affect HR, nor did it alter alfentanil’s effect (data not shown). No volunteer met criteria for treatment for bradycardia. Alfentanil, neostigmine and their combination had no effect on blood pressure, and no volunteer met criteria for treatment for increased or decreased blood pressure.

Neurochemical and drug analyses. At the end of the stepped alfentanil infusion (final target either 100 or 200 ng/ml in plasma), there was detectable alfentanil in the CSF in all volunteers. CSF alfentanil concentrations (mean = 3.7 ± 0.3 ng/ml, range 0.7–8.9 ng/ml) represented 4.0 ± 0.2% of the simultaneously measured plasma alfentanil concentration. Drug treatments did not affect CSF norepinephrine concentrations (data not shown). In contrast, i.v. alfentanil alone increased CSF ACh concentrations (P < 0.01). There was no significant relationship between plasma alfentanil concentration and increase in ACh when alfentanil was administered alone, but when it was administered with intrathecal neostigmine, there was a significant, positive interaction between plasma alfentanil and change in ACh (P < 0.05; fig. 7).

Discussion

Although intrathecal neostigmine injection has previously been reported to reduce i.v. opioid consumption after surgery (Lauretti and Lima, 1996), this is the first detailed examination of the interaction between these therapies. The results support data obtained in animals, which demonstrate a spinal cholinergic mechanism of systemic opioid analgesia.
Fig. 7. Effect of alfentanil and neostigmine on change in ACh concentrations in CSF. For each volunteer, the effect of alfentanil was determined as the difference between the CSF ACh concentration after the i.v. alfentanil infusion and the CSF ACh concentration before infusion. Alfentanil in the absence of intrathecal neostigmine produced a significant (P < .01) plasma alfentanil concentration-independent increase in CSF ACh (■, dotted regression line). Intrathecal neostigmine produced a dose-dependent increase in alfentanil’s effect (P < .01 for interaction) and resulted in an alfentanil concentration-dependent increase in CSF ACh (○ = 50 µg, □ = 100 µg, △ = 200 µg, solid regression line for entire neostigmine-alfentanil data set). * P < .05 compared with alfentanil alone.

(Chiang and Zhuo, 1989) but also demonstrate that neostigmine enhances a bothersome side effect of opioids, nausea.

Analgesia. Lumbar injection of neostigmine in dextrose increased CSF ACh concentrations and produced greater analgesia in the foot than in the hand, a result consistent with previous reports in volunteers (Hood et al., 1995b) and with a dermatomally restricted pattern of analgesia after spinal administration. It was assumed in the current study, as previously shown in volunteers (Hood et al., 1995b), that neostigmine’s effect was maximal within 60 min of injection and stable for the next 60 min.

That i.v. alfentanil increased CSF ACh concentrations corroborated a single case report of i.v. opioid-induced increases in CSF ACh concentrations in a human (Bouaziz et al., 1996). More precise experiments in animals are consistent with a spinal cholinergic mediation of systemic opioid analgesia. For example, antinociception from systemic opioid administration in rats is inhibited by intrathecal administration of atropine (Chiang and Zhuo, 1989), and i.v. morphine injection in sheep causes a dose-dependent increase in ACh concentrations in CSF and in microdialysate samples from dorsal, but not ventral, spinal cord (Bouaziz et al., 1996). These are the first systematic data in human testing that support this spinal cholinergic mechanism of i.v. opioid analgesia. The ratio of CSF alfentanil concentration to plasma alfentanil concentration was constant (4 ± 0.2%), providing the first direct measure of the relationship of the amount of alfentanil in human CSF during i.v. computer-controlled administration to a presumed stable plasma concentration. This is not to say, however, that the CSF concentration of alfentanil is at a stable level at this time, because repeated CSF sampling was not performed.

Neostigmine enhanced alfentanil-induced analgesia in the current study, a result that further supports the opioid-cholinergic interaction. The quantitative analysis of this interaction to testing in the foot and in the hand is revealing. First, neostigmine enhanced alfentanil analgesia to pain testing in the foot in an additive manner, where neostigmine alone had a large effect. In the hand, where neostigmine alone had a minor effect, this interaction was synergistic. Because the nature of drug interaction (additive vs. synergistic) varies with the ratio at which they are combined (Tallarida et al., 1989), and because one would anticipate a lesser concentration of neostigmine in cerebral than in lumbar CSF after lumbar injection, these data suggest that a lower neostigmine/alfentanil ratio than 100 µg:100 ng/ml (plasma) might be more likely to demonstrate synergy in the foot. Second, the lowest dose of intrathecal neostigmine, 50 µg, produced a large reduction in the apparent alfentanil EC50 to pain testing in the foot, and relatively little further reduction occurred with larger neostigmine doses. This is consistent with preliminary data in postoperative patients, in which intrathecal neostigmine, over a dose range of 10 to 100 µg, produced a dose-independent 40% to 50% reduction in i.v. morphine use.

Respiratory depression. As previously observed in volunteers, i.v. alfentanil administration produces respiratory depression (Eisenach et al., 1993; Hill et al., 1990). Because this is the most dangerous potential side effect of i.v. opioids, interaction of other drugs in this side effect is particularly important. Animal studies suggest that i.v. administration of cholinesterase inhibitors that cross the blood-brain barrier can antagonize opioid-induced respiratory depression (Elmaleh et al., 1991; Willette et al., 1987). We did not measure respiratory drive directly in the current study but rather used crude measures (end-tidal CO2 and SpO2) that are utilized clinically to define meaningful depression of resting ventilation in postoperative patients. Using these measures, we failed to observe antagonism of alfentanil-induced respiratory depression by lumbar intrathecal neostigmine injection in the current study, perhaps because inadequate concentrations of neostigmine reached brainstem sites of respiratory control. Nonetheless, intrathecal injection of neostigmine, though it resulted in more sedation when combined with i.v. alfentanil, had no effect on alfentanil-induced respiratory depression. Indeed, we hypothesize that because intrathecal neostigmine reduced the EC50 of alfentanil for analgesia by half or more, clinical studies will demonstrate reduced risk of respiratory depression postoperatively when intrathecal neostigmine is utilized.

Nausea. Both opioid and cholinergic agents can produce nausea by action in the chemotrigger zone of the upper brainstem. Nausea was severe in these volunteers who received large doses of i.v. alfentanil and intrathecal neostigmine. Preliminary data in postoperative patients demonstrate reduced nausea in patients receiving low doses (≤50 µg) of intrathecal neostigmine, probably because of the reduction in these patients’ use of morphine (Lauretti and Lima, 1996). Thus it would appear that the neostigmine dose may need to be reduced to this level in order to avoid enhancement of this particularly bothersome side effect of neostigmine and opioids.

Other side effects. Both i.v. opioids and intrathecal neostigmine have been previously demonstrated to produce sedation. Neostigmine failed to produce sedation in the cur-
rent study, which is consistent with the smaller dose admin-
istered, but it did enhance alfentanil-induced sedation. As
noted above, this sedation did not result in more respiratory
depression. Dose-related subjective leg weakness and de-
creased deep-tendon reflexes from neostigmine have also
been observed in volunteers (Hood et al., 1995b) although
their etiology is uncertain. Sedation and leg weakness were
significantly correlated in volunteers in the current study
(Spearman rank correlation \( P < 0.01 \); mixed-effects regression
\( P < 0.01 \)), which is consistent with the hypothesis that the
volunteers’ self-assessment of subjective weakness was more
extreme during periods of alfentanil-induced increases in
sedation. Hemodynamic effects of i.v. opioids or intrathecal
neostigmine are minor, with mild sympatholysis from opioids
in large doses and sympathetic activation from neostigmine
in large doses only, as evidenced by minimal changes in blood
pressure and HR in the current study.

**Neurochemical and drug analyses.** Intravenous mor-
phine has been shown to increase CSF norepinephrine and
ACh in sheep and in one volunteer (Bouaziz et al., 1996).
In the current study, CSF ACh increased after i.v. alfentanil
and further increased in combination with intrathecal
neostigmine, but CSF norepinephrine did not increase. The
lack of repeated CSF sampling in the current study may have
diminished our ability to see an effect on CSF norepineph-
rine, because the time of peak increase in norepinephrine
differs between ACh and norepinephrine (Bouaziz et al.,
1996).

Finally, it should be noted that these data in volunteers do
not necessarily predict the interaction of these therapies in
patients with pain. Pain itself activates bulbospinal descend-
ing inhibitory pathways that may activate spinal cholinergic
neurons. Thus acute noxious stimulation increases CSF ACh
concentrations (Eisenach et al., 1996), and intrathecal
neostigmine analgesia is more profound in the acute postop-
erative period than later in sheep (Bouaziz et al., 1995).

Pain-induced spinal cholinergic activation may explain the
40% to 50% reduction in i.v. opioid use in postoperative
patients receiving 10 \( \mu \)g neostigmine, in contrast to the mini-
mal effect of 50 \( \mu \)g of neostigmine in volunteers without
ongoing pain (Hood et al., 1995b; Laurer tti and Lima, 1996).

Similarly, side effects from i.v. and intrathecal opioid admin-
istration are greater in volunteers without ongoing pain than
in patients (Bailey et al., 1993), and it is quite conceivable
that analgesia from i.v. opioids in postoperative patients is
more enhanced by intrathecal neostigmine injection than are
side effects.

In conclusion, both intrathecal neostigmine and i.v. alfent-
nal increase CSF ACh concentrations and produce analge-
sia in healthy volunteers, and the lowest neostigmine dose
studied, 50 \( \mu \)g, reduces the \( \text{EC}_{50} \) of i.v. alfentanil by 50%.

Neostigmine does not enhance respiratory depression in-
duced by i.v. alfentanil, as inferred from end-tidal \( \text{CO}_2 \) and
\( \text{SpO}_2 \) measurements, although it does enhance alfentanil-
induced sedation. Neostigmine also enhances alfentanil-
induced nausea, which could limit the clinical utility of this
combination. However, because there may be significant dif-
ferences in analgesic drug efficacy and side effects between
volunteers without ongoing pain and patients, this interac-
tion deserves clinical investigation.

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