Interaction between Intrathecal Neostigmine and Epidural Clonidine in Human Volunteers

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Background: α_2-Adrenergic agonists are thought to produce analgesia, in part, by activating spinal acetylcholine release. The purpose of the current study was to examine the interaction between intrathecal neostigmine and epidural clonidine for analgesia and side effects in humans.

Methods: A total of 58 volunteers received an intrathecal injection of 5% dextrose in normal saline (D5NS) or neostigmine (50, 100, or 200 μg in D5NS), followed in 1 h by epidural saline or clonidine (computer-controlled infusion targeted to 50, 100, 200, or 400 ng/ml in cerebrospinal fluid) using an isobolographic design. Visual analog scale pain to a noxious cold stimulus, nausea, weakness, sedation, and other safety variables was measured before and at specified intervals after drug administration.

Results: The first 21 volunteers randomized to receive intrathecal hyperbaric neostigmine rather than D5NS received the drug while in the sitting position, and had none-to-minimal analgesia 1 h later. The remaining volunteers received the drug while in the lateral position, and demonstrated dose-dependent analgesia in the foot 1 h later. Epidural clonidine also caused dose-dependent analgesia. The combination of neostigmine and clonidine resulted in an additive enhancement for analgesia, but no enhancement of each drug's side effects, and a reduction in clonidine-induced hypotension. Neostigmine injected into subjects in the lateral position diminished clonidine-induced reductions in blood pressure and plasma norepinephrine.

Conclusion: These results support enhancement of α_2-adrenergic analgesia by intrathecal neostigmine, but do not demonstrate synergy, as observed in animals. Lack of enhancement of side effects suggests this combination may be clinically useful. (Key words: Analgesia, epidural: clonidine. Analgesia, spinal: neostigmine. Neurotransmitters: acetylcholine.)

SPINALLY mediated analgesia can be produced by several mechanisms. Local anesthetics cause nonspecific axonal blockade. More specific blockade of nociception may be accomplished by injection of direct agonists, such as opioids or α_2-adrenergic agonists, which stimulate receptors involved in nociceptive processing in the spinal cord. Unlike these direct-acting agonists, neostigmine inhibits the breakdown of an endogenous neurotransmitter, acetylcholine, which was shown to cause analgesia in animals.[1–3] Inhibiting breakdown of acetylcholine has several theoretical benefits, if one could selectively stimulate acetylcholine release only at areas of therapeutic interest, but also causes side effects at other sites of action unrelated to analgesia (motor weakness, nausea, sympathetic activation).

After extensive preclinical toxicity testing,[4,5] an open-label, dose-ranging trial of intrathecal neostigmine was performed in humans.[6] This revealed dose-dependent analgesia from neostigmine alone, but also dose-dependent side effects, chiefly nausea and motor weakness.

The effects of stimuli that cause the release of acetylcholine in the spinal cord may be augmented by inhibiting acetylcholine metabolism. Several circumstances are thought to stimulate this spinal acetylcholine release: pain,[7] systemic opioids,[8] and intraspinal α_2-adrenergic agonists.[9] In animals and humans, intrathecal or epidural administration of the α_2-adrenergic agonist, clonidine, increases acetylcholine in cerebrospinal fluid (CSF),[9] and intrathecal neostigmine synergistically enhances antinociception from clonidine in animals.[2] The purpose of this study was to assess, in humans, the analgesia and side effects of intrathecal neostigmine combined with epidural clonidine infused to targeted CSF concentrations. A secondary aim was to examine the effects of these agents

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on CSF concentrations of acetylcholine, norepinephrine, and cyclic guanosine monophosphate (cGMP) to indirectly test the hypothesis that these agents act *via* acetylcholine release and perhaps *via* stimulation of nitric oxide (NO) synthase. The rationale for studying a measure of NO activity is previous work in animals demonstrating stimulation of NO synthesis from increased concentrations of acetylcholine after intrathecal neostigmine injection.[10,11]

Methods

The Clinical Research Practices Committee approved all aspects of this study. Approved advertisements for study volunteers were placed on public bulletin boards in the medical center and the student union of the affiliated university. Prospective volunteers who could not tolerate immersion of their hand or foot for 60 s in stirred ice water or who rated their pain < 6 cm on a 10-cm visual analog scale (VAS) for pain were excluded from study participation. This procedure excluded those individuals who did not find ice-water immersion of their hand or foot particularly painful or those who found the test too painful to tolerate for 60 s. Only healthy adults (ASA physical status 1) taking no medications were included. Written, informed consent was obtained and serum human chorionic gonadotropin concentrations were determined in all female volunteers to exclude pregnancy.

Volunteers reported to the General Clinical Research Center at 7 AM, having had nothing to eat or drink since midnight. Immersion of the hand and foot in stirred ice water was performed and VAS scores obtained. Two individuals were excluded from study because their VAS pain scores were less than 5 on the morning of planned study, despite having VAS scores > 6 on their initial screening test. In each of the remaining 58 studies, a peripheral intravenous catheter was inserted in one arm for infusion of lactated Ringer's solution at 50–100 ml/h, and a second intravenous catheter was inserted into the contralateral arm, capped, and used for blood sampling throughout the study.

General Study Design

After obtaining baseline measures, volunteers were positioned sitting (first 32 volunteers) or lateral (last 26 volunteers). The position was changed to lateral in the last 26 volunteers after interim analysis revealed minimal analgesia from hyperbaric neostigmine in-

jected while the subjects were sitting. Regardless of volunteer position, an epidural needle was inserted at the L3–4 or L4–5 interspace, and a #25 or 27 spinal needle was inserted through the epidural needle until clear CSF was obtained. After intrathecal drug injection in a 1.0 ml volume for 15 s, the spinal needle was withdrawn, an epidural catheter inserted 3–5 cm beyond the tip of the epidural needle, the epidural needle withdrawn, and the catheter taped in place. Volunteers were then positioned supine for at least 15 min, after which the head of the bed could be elevated by as much as 30°.

Seventy minutes after intrathecal injection, a computer-controlled bolus infusion of epidural saline or clonidine was begun, using a Harvard pump (CR Bard, North Reading, MA) and a modified STANPUMP algorithm. Seventy minutes later, the target concentration of CSF clonidine was set to 0, maintained at the same level, or increased to the next level (see below). Seventy minutes later, the epidural infusion was discontinued and intravenous location of the epidural catheter tip was excluded by injecting 20 µg epinephrine in 4 ml normal saline through the epidural catheter and monitoring the heart rate for 2 min for increased heart rate (>15 beats/min change). A second sample of CSF was obtained 120–180 min after neostigmine injection (see below). After completion of the study, the volunteer was allowed to eat and then rested until drug side effects were sufficiently diminished to safely discharge the volunteer into the care of another adult.

Drug Administration

Drugs were administered using an isobolographic design for study of drug interaction, with doses prospectively and randomly assigned based on a computer-generated random number table. The research nurse who obtained outcome variables, including VAS scores, was blinded to study drug. For intrathecal drug injection, volunteers were randomized to receive vehicle (5% dextrose in normal saline) or neostigmine (50, 100, or 200 µg), diluted in 5% dextrose in normal saline into a total volume of 1 ml (2 volunteers received 1.5 ml neostigmine solution). The maximum dose of neostigmine was chosen, based on previous study, to provide maximal analgesia and minimal-moderate risks for nausea/vomiting.

Because we did not have investigational new drug approval for intrathecal administration of clonidine, we infused clonidine epidurally to targeted CSF concentrations, based on previous pharmacokinetic/phar-

macodynamic studies in volunteers.[12] The clonidine/ placebo infusion was either normal saline or targeted to 50, 100, 200, or 400 ng/ml clonidine CSF concentration. The initial clonidine CSF target was selected randomly to provide all possible drug combinations with neostigmine in the first hour of infusion. The one exception was the 400 ng/ml clonidine CSF target, which was only administered in the second hour of infusion after intrathecal placebo.

Dose responses were thus constructed for neostigmine alone (measures obtained 1 h after intrathecal neostigmine injection and before initiating clonidine infusion), clonidine alone (measures obtained 2 and 3 h after intrathecal saline injection, which was the end of the first and second hours of epidural clonidine infusion, respectively), and the combination (measures obtained 2 and 3 h after intrathecal neostigmine injection, which was the end of the first and second hours of epidural clonidine infusion, respectively). The purpose of the second hour of clonidine infusion was twofold: first, to allow study of two escalating doses of clonidine in volunteers receiving intrathecal saline, thereby reducing the number of volunteers required, and second, to test the constancy of neostigmine and clonidine analgesia throughout this time period.

Drugs were administered in a constant ratio of neostigmine dose: clonidine CSF concentration in an isobolographic design. These were 50 μg neostigmine:50 ng/ml clonidine, 100 μg neostigmine:100 ng/ml clonidine, and 200 μg neostigmine:200 ng/ml clonidine. We chose to use raw total epidural clonidine dose (for the first hour, this was 52 μg for the 50 ng/ml target; 104 μg for the 100 ng/ml target; and 209 μg for the 200 ng/ml target) in the isobolographic analysis, although the analysis results were not changed when targeted CSF concentration was used.

Outcome Variables

Analgesia (VAS pain report) was determined after 60-s immersion of the foot and hand (randomized order and randomized side [left *versus* right]) in stirred ice water. This was performed before intrathecal injection, approximately 60–65 min after intrathecal injection, and approximately 60–65 min after each of the two targeted epidural infusions.

The following variables were obtained at baseline, and then between 50 and 65 min after intrathecal injection and after each of the two targeted epidural infusions: oxyhemoglobin saturation by pulse oximetry (SpO_2), end-tidal CO_2, upper and lower extremity light

touch and cold sensation, a screening neurologic examination of extremities, venous blood sampling, VAS sedation (anchors: wide awake, as sleepy as could be), VAS nausea (anchors: no nausea, as nauseated as could be), and VAS leg weakness (anchors: no weakness, as weak as could be). The screening neurologic examination of the upper and lower extremities consisted of qualitative assessment (parameter increased, decreased, or similar to baseline) of deep tendon reflexes, joint extension strength of ankle, knee, and elbow, sensation of light touch by cotton wisp, and cold sensation to an alcohol pad swiped across the skin. In addition, at each time period measurement, subjective comments were elicited concerning general feelings, side effects such as cramping, salivation, or visual blurring, and perception of leg sensations. The number of vomiting episodes was tabulated for each 60-min time period. At the conclusion of the experiment, the volunteer answered a short written questionnaire about the occurrence of sexual feelings, unusual feelings in the genital area, sensation of "gas" or bloating, and feelings of urinary urgency or hesitancy.

To determine the effect of drug treatments on acetylcholine, norepinephrine, and cGMP, 1.5 ml CSF was sampled at two times: through the spinal needle just before drug injection and, by a second insertion of a #25 or 27 spinal needle, after drug administration. The second sample was obtained after either the first or second hour of epidural clonidine infusion, and therefore represented the effect of clonidine alone (in the intrathecal placebo group) or the combination of neostigmine and clonidine. In addition, venous blood was sampled at hourly intervals through the study and analyzed for plasma norepinephrine. All samples were immediately frozen on powdered dry ice and kept at −70°C until analysis.

Norepinephrine 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 and an absolute detection limit of 12 fmol.[13] Acetylcholine concentrations were determined by a different high pressure liquid chromatography-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.[9] Cyclic guanosine monophosphate concentrations were determined by radioimmunoassay, with an interassay coefficient of variation of 4% and a detection limit of 50 fmol.[14]

Side Effects and Treatment. Nausea that persisted into the recovery period was treated with 0.5 mg droperidol, repeated 1–2 times, followed by phenergan 12.5–25 mg intravenously and/or 2–4 mg ondansetron intravenously. Volunteers who experienced postdural puncture headaches that did not resolve within 24 h were treated by epidural blood patch using 10–20 ml blood. Volunteers were contacted by phone daily for 1 week, and at 2 weeks, 1 month, and 6 months after completing the study. At these times, patients were questioned concerning neurologic symptoms and headache, and unsolicited questions were answered.

Drugs. Droperidol was obtained from Janssen Pharmaceutica (Titusville, NJ). Phenergan was obtained from Wyeth-Ayerst Laboratories (Philadelphia, PA). Ondansetron was obtained from Ceremax (Research Triangle Park, NC). Neostigmine was obtained under investigational new drug approval by the Food and Drug Administration in preservative-free saline from International Medication Systems, (El Monte, CA). Neostigmine from this same commercial source had been used in preclinical toxicity studies.

Statistics. Unless otherwise stated, data are presented as mean ± SEM. The experiment was analyzed using a repeated measures design. Primary outcome parameters and possible covariates were assessed. Primary outcome parameters included systolic, diastolic, and mean arterial blood pressure, heart rate, respiratory rate, end-tidal CO_2, and VAS scores for nausea, sedation, leg weakness, hand pain, and foot pain. In addition, two derived pain variables—percent maximum possible effect (%MPE) for hand pain and foot pain—were included in the analysis, calculated as:

%MPE = (observed)/(baseline)*100

Simple analyses of variance were performed to determine which covariates statistically influenced each outcome variable. The covariates were age, height, weight, and sex. To allow for curvilinear relations, quadratic as well as linear models were fit.

A regression approach was used to test for drug interactions. Each drug-response curve was statistically modeled alone, and then a larger model containing terms for both drugs was developed. Linear and quadratic models were analyzed using mixed model multiple regression. Because of the small number of drug doses tested,[3,4] the quadratic model was elected to test curvilinear dose-response curves.

A mixed model regression was performed comparing the time effects of patients receiving no neostigmine to patients receiving neostigmine. Clonidine CSF target

interactions between the first and second hour of infusion were also included as effects in the model. In addition, a mixed models analysis of variance using maximum likelihood estimation was performed with no covariates. Covariates were not needed because each patient acts as his own control, so between-patient covariate effects are confounded with patient effects. $P < 0.05$ was considered significant.

Results

Volunteers were 29 ± 1.1 yr old, 169 ± 2 cm in height, and 75 ± 3.1 kg in weight. Data from two volunteers were excluded from analysis: one for evidence of intravascular location of the epidural catheter after testing with epinephrine (200 μg neostigmine/200 ng/ml clonidine group), and one for wide inconsistency in pain report who was excluded by posthoc statistical analysis (50 μg neostigmine/50 ng/ml clonidine group). Treatment groups did not differ in concentrations of neurochemicals in CSF before drug injection.

Analgesia

Patient position significantly affected analgesia from intrathecal neostigmine injection (fig. 1). For this reason, only clonidine-neostigmine analgesia data obtained with volunteers in the lateral position were used for interaction analysis. Visual analog scale pain scores did not change during the second hour of epidural clonidine infusion in those volunteers randomized to have identical infusion targets for both hours of infusion (data not shown).

Each drug alone and their combination provided dose-related analgesia in the foot (fig. 2). Regression analysis revealed an ED50 of clonidine to be 248 ± 110 μg (mean ± SD), neostigmine to be 183 ± 85 μg, and the combination to be 230 ± 147 μg. Isobolographic analysis confirmed their interaction to be additive (fig. 2, insert). In contrast, effects on the report of pain in the hand, although statistically significant, were minor (fig. 3).

Effect on Cerebrospinal Fluid Acetylcholine, Neostigmine, and Cyclic Guanosine Monophosphate

Epidural clonidine alone produced a dose-related increase in CSF acetylcholine, and combination with intrathecal neostigmine resulted in a further increase in acetylcholine only when neostigmine was injected
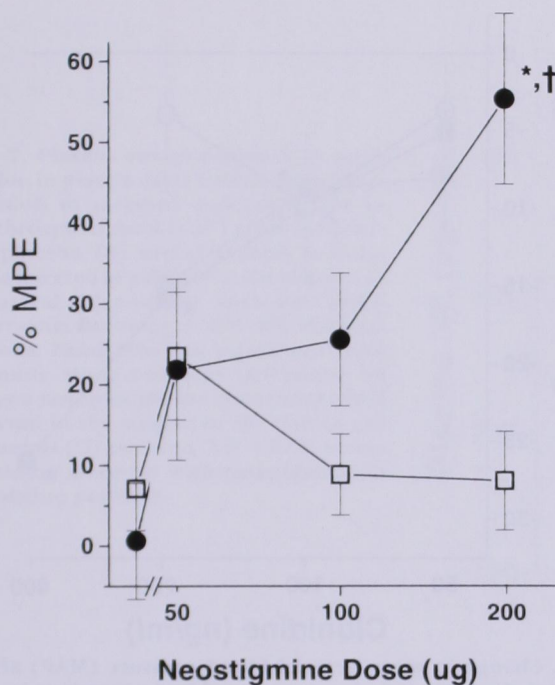
Fig. 1. Effect of position on analgesic effect in the foot of intrathecal neostigmine. Percent maximum possible effect (%MPE) for analgesia 60 min after injection of intrathecal neostigmine with volunteers in the sitting (□) or lateral (●) position. Each data point represents mean ± SEM of 5–8 observations. * $P < 0.05$ *versus* no neostigmine; † $P < 0.05$ *versus* sitting position.
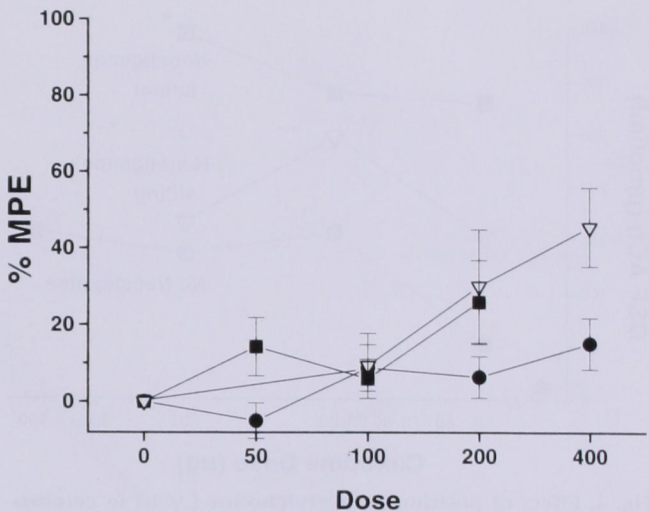
Fig. 3. Analgesia in the hand, expressed as percent maximum possible effect (%MPE) from intrathecal neostigmine alone (■), epidural clonidine alone (●), or their combination (▽). Dose expressed as micrograms (neostigmine), ng/ml in cerebrospinal fluid (clonidine) or the numerical addition of these two (combination). Each data point represents the mean ± SEM of 5–8 observations.

while the subjects were in the lateral position (fig. 4). Lumbar CSF neostigmine concentrations 2 h after injection were similar with injection in subjects in the lateral or sitting position (median of 30, 34, and 43 ng/ml after injection of 50, 100, and 200 μg, respectively, from injections while subjects were in

Fig. 2. Analgesia in the foot, expressed as percent maximum possible effect (%MPE) from intrathecal neostigmine alone (■), epidural clonidine alone (●), or their combination (▽). Dose expressed as micrograms for each drug alone or the numerical addition of these two (combination). Each data point represents the mean ± SEM of 5–8 observations. *Inset:* Isobologram at the ED50 level, demonstrating additivity of the interaction.
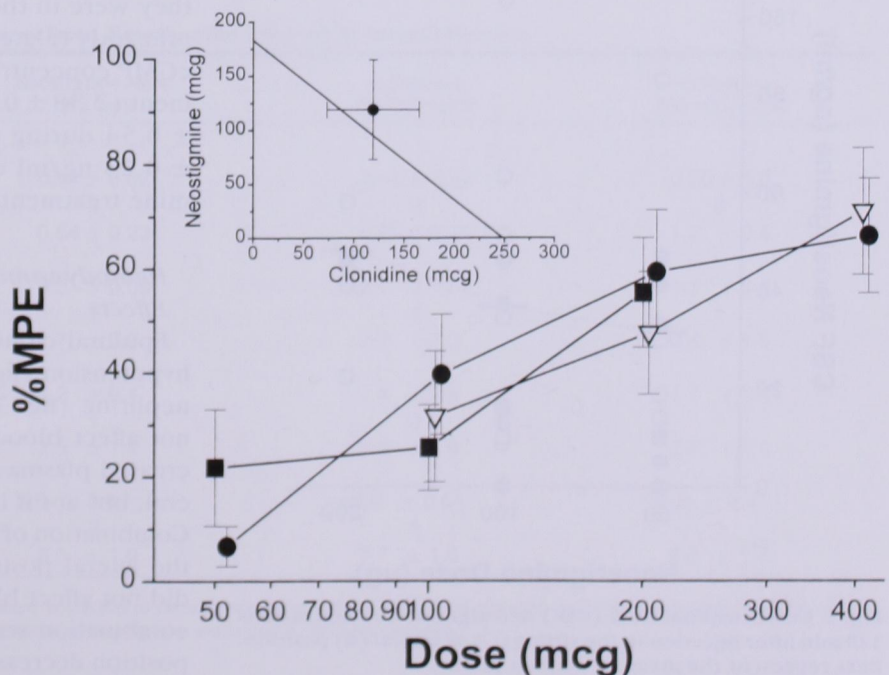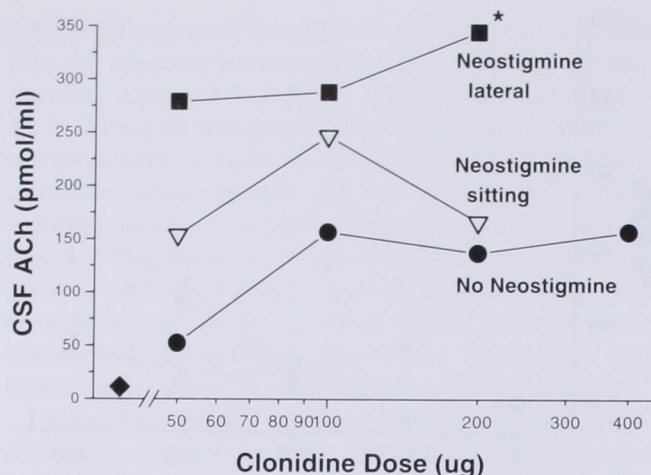
Fig. 4. Effect of position on acetylcholine (ACh) in cerebrospinal fluid (CSF). Cerebrospinal fluid acetylcholine *versus* targeted CSF clonidine concentrations in patients not receiving intrathecal neostigmine (●) and in those receiving neostigmine injected while in the sitting (▽) or lateral (■) position. Each data point represents the median of 4–8 observations. Clonidine in all groups increased acetylcholine significantly ($P < 0.001$). Compared with no neostigmine, only the group receiving neostigmine in the lateral position demonstrated increased CSF acetylcholine (*$P < 0.05$ *vs* no neostigmine).

Fig. 6. Change in mean arterial blood pressure (MAP) after epidural clonidine infusion to targeted concentrations in cerebrospinal fluid (CSF) after intrathecal placebo (■) or intrathecal neostigmine injected in subjects in the sitting (▲) or lateral (○) position. Each data point represents the mean ± SEM of 5–8 observations. * $P < 0.05$ *versus* baseline values.
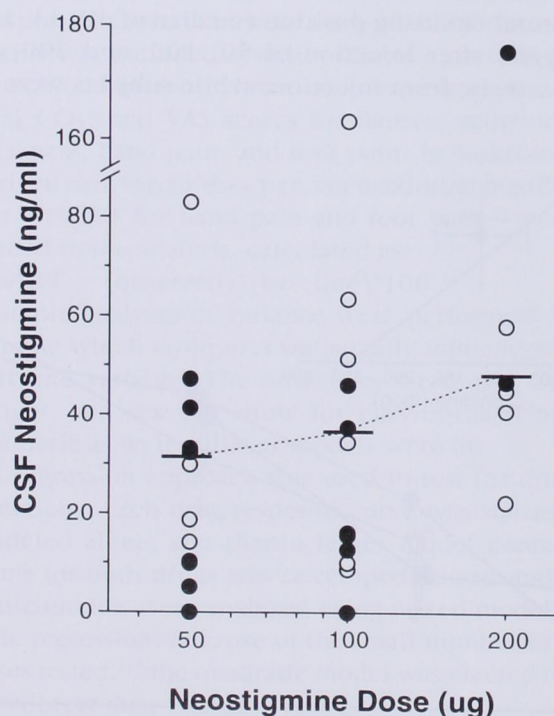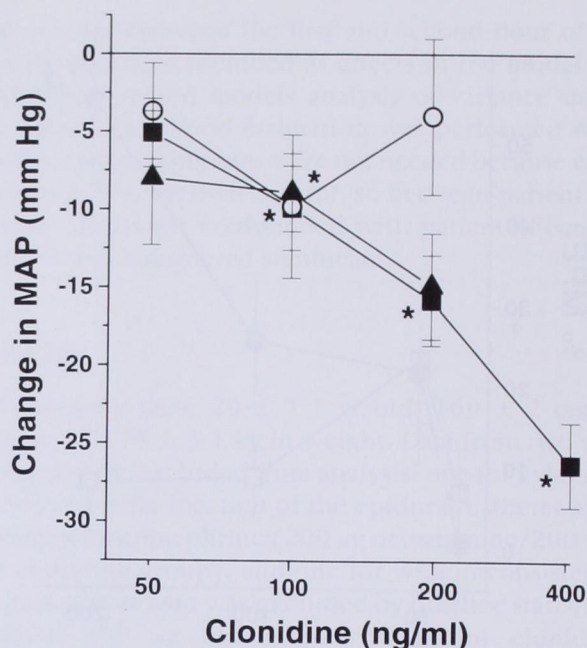

Fig. 5. Cerebrospinal fluid (CSF) neostigmine concentrations 120 min after injection in the sitting (○) or lateral (●) position. Bars represent the mean of all data points.

the sitting position, and 33, 27, and 47 ng/ml while they were in the lateral position), and were not significantly related to dose (fig. 5). Cerebrospinal fluid cGMP concentrations were unaffected by drug treatment (2.38 ± 0.21 ng/ml before drug injection, 2.35 ± 0.54 during clonidine treatment alone, and 2.25 ± 0.23 ng/ml during combined clonidine–neostigmine treatment).

Hemodynamic and Plasma Norepinephrine Effects

Epidural clonidine alone produced dose-dependent hypotension (fig. 6) and reduction in plasma norepinephrine (fig. 7). Intrathecal neostigmine alone did not affect blood pressure (data not shown), and increased plasma norepinephrine, if injected in the lateral, but not if injected in the sitting position (fig. 7). Combination of clonidine and neostigmine injected in the lateral position at the highest dose combination did not affect blood pressure, whereas the same dose combination with neostigmine injected in the sitting position decreased blood pressure similar to clonidine

Fig. 7. Plasma norepinephrine concentration in plasma after epidural clonidine infusion to targeted concentrations in cerebrospinal fluid (CSF) after intrathecal placebo (■) or intrathecal neostigmine injected in subjects in the sitting (▲) or lateral (○) position. Each data point represents the mean ± SEM of 5–8 observations. *Inset:* Effect of intrathecal neostigmine alone (without clonidine) on plasma norepinephrine concentration if injected in the subject in the sitting (▲) or lateral (○) position. * $P < 0.05$ *versus* clonidine alone or with neostigmine in the sitting position.
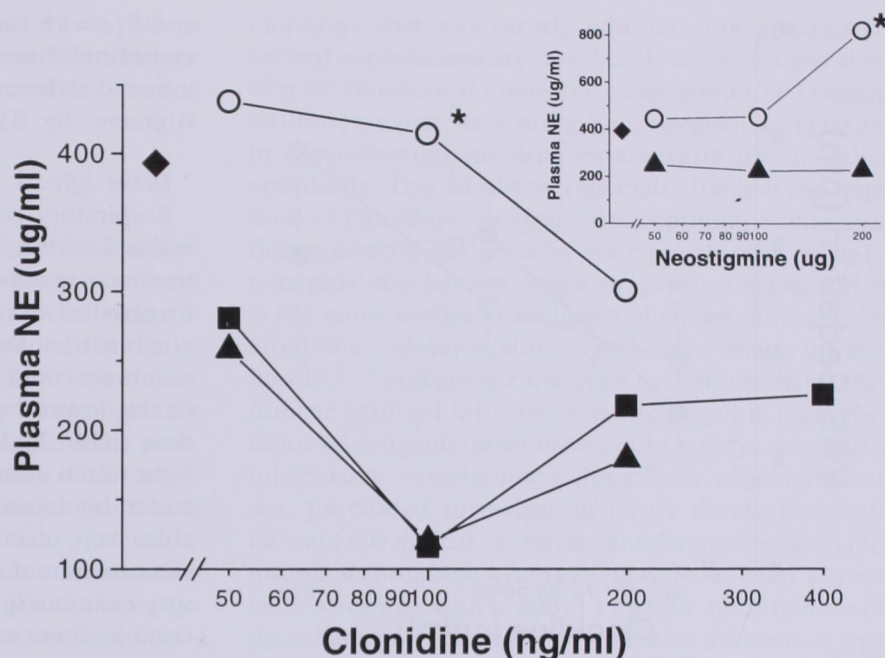
alone (fig. 6). Similarly, the reduction in plasma norepinephrine produced by clonidine was unaffected by neostigmine injected in the sitting position, but was antagonized by neostigmine injected in the lateral position.

Effect on Nausea, Sedation, and Weakness

Intrathecal neostigmine (lateral position injection), but not epidural clonidine or intrathecal injection in the sitting position, caused nausea and weakness, and these effects were not altered by combination with clo-

Table 1. Nausea and Lower Extremity Weakness from Neostigmine Injected in Lateral Position

Neostigmine Dose	Neostigmine Alone (60 min)	+ Clonidine (50–200 ng/ml)	+ Clonidine (50–400 ng/ml)
Placebo (n = 11)			
VAS nausea (cm)	0.14 ± 0.02	0.14 ± 0.02	0.20 ± 0.0
Individuals vomiting	0	0	0
VAS weakness (cm)	0.54 ± 0.23	0.66 ± 0.24	1.2 ± 0.4
50 μg (n = 6)			
VAS nausea (cm)	0.3 ± 0.09	2.0 ± 1.7	1.1 ± 0.4
Individuals vomiting	1	1	1
VAS weakness (cm)	1.7 ± 0.9	2.7 ± 1.0	2.4 ± 1.0
100 μg (n = 6)			
VAS nausea (cm)	1.2 ± 0.4	1.4 ± 0.5	1.1 ± 0.4
Individuals vomiting	1	0	1
VAS weakness (cm)	2.5 ± 1.4	2.9 ± 1.6	2.8 ± 1.1
200 μg (n = 8)			
VAS nausea (cm)	3.5 ± 0.6	6.0 ± 0.6	4.2 ± 0.4
Individuals vomiting	2	5	3
VAS weakness (cm)	5.3 ± 1.0	7.7 ± 1.0	6.3 ± 1.8

Values are as mean ± SEM. For clarity data are presented according to neostigmine dose without separating clonidine target concentration. Analysis reveals significant dose-dependent nausea and weakness from intrathecal neostigmine unaffected by epidural clonidine.
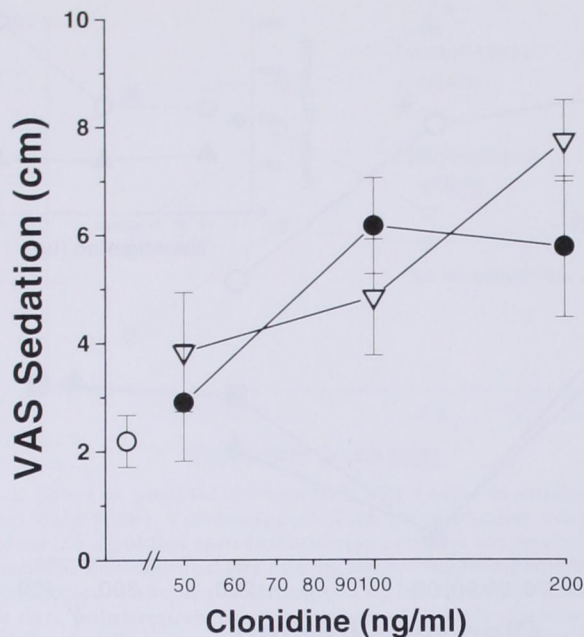
VAS = visual analog scale.

Fig. 8. Visual analog scale (VAS) sedation in volunteers receiving epidural clonidine alone (●) or with intrathecal neostigmine (▽). Each data point represents the mean ± SEM of 5–8 observations. Groups do not differ.

nidine (table 1). Intrathecal neostigmine (lateral position injection) produced mild sedation alone (5.2 ± 1.3 cm after the largest dose in the lateral position, 2.1

± 0.7 cm in the sitting position), whereas epidural clonidine caused intense sedation (fig. 8). Clonidine-induced sedation was not affected by intrathecal neostigmine (fig. 8).

Other Effects

Respiratory rate, heart rate, SpO_2, and $ETCO_2$ remained within 10% of baseline values, regardless of treatment group. Apparent muscle strength, as assessed by physical examination, was unaffected by any drug combination. Deep tendon reflexes, awareness of light touch, and cold sensation were significantly depressed in the lower extremities and related to neostigmine dose (table 2). Upper extremity deep tendon reflexes, light touch awareness, and cold sensation were unaffected by neostigmine dose. The above outcome variables were unaffected by clonidine dose as reflected by examination of the upper and lower extremities. Sensory examination effects at time points assessing drug combinations were statistically related to the dose of neostigmine in the drug combination.

Seven volunteers experienced symptoms of postdural puncture headache. Three volunteers received an epidural blood patch, with resolution of their headache. There were no other long-term symptoms or side-effects attributable to study participation. One volunteer in the intrathecal placebo group experienced viral meningitis 10 days after completing the study. This vol-

Table 2. Neurologic Examination in Lower Extremities with Neostigmine in Lateral Position

Neostigmine Dose	Neostigmine Alone (60 min)	+ Clonidine (50–200 ng/ml)	+ Clonidine (50–400 ng/ml)
Placebo (n = 11)			
No. decreased DTRs	2	2	6
No. decreased light touch sense	1	2	2
No. with decreased cold sense	1	6	4
50 µg (n = 6)			
No. decreased DTRs	0	1	0
No. decreased light touch sense	1	1	0
No. with decreased cold sense	0	1	0
100 µg (n = 6)			
No. decreased DTRs	3	5	5
No. decreased light touch sense	0	3	3
No. with decreased cold sense	3	4	1
200 µg (n = 8)			
No. decreased DTRs	4	4	4
No. decreased light touch sense	2	3	3
No. with decreased cold sense	4	3	4

Values are mean ± SEM. For clarity data are presented according to neostigmine dose without separating clonidine target concentration. Analysis reveals significant dose-dependent effects of intrathecal neostigmine and epidural clonidine alone but no significant interaction.

DTRs = deep tendon reflexes.

unteer related a history of being exposed to a patient who had viral meningitis, approximately 7 days before experiencing his symptoms. He subsequently recovered completely from his viral meningitis.

Discussion

Analgesia from intraspinally administered opioids and α_2-adrenergic agonists has been extensively examined clinically, whereas the effects of intrathecal neostigmine are just beginning to be explored. In animals, opioids and α_2-adrenergic agonists interact synergistically after intraspinal administration,[15] although this interaction is weakly synergistic or additive in postoperative patients.[16] This is the first study of the interaction between an α_2-adrenergic agonist and intrathecal neostigmine in humans.

There are several reasons to expect an enhancement of α_2-adrenergic agonist-induced analgesia by neostigmine. For one, epidural clonidine increases acetylcholine in CSF of humans, as observed in the current study and previously.[17] Similarly, intrathecal clonidine increases acetylcholine in CSF and dorsal horn microdialysis samples in animals,[9,18] an effect blocked by idazoxan, the specific α_2-adrenergic antagonist. For another, intrathecal neostigmine enhances antinociception from α_2-adrenergic agonists in sheep[7,9] and rats.[2] Similarly, antinociception from α_2-adrenergic agonists in rats is inhibited by atropine,[19] suggesting a cholinergic mechanism. Although the interaction between intrathecal neostigmine and clonidine is synergistic in rats,[2] this is the first study of this interaction in humans.

Isobolographic analysis is a robust method to study drug interactions, because it makes no assumptions regarding the shape of the dose-response relations.[20] However, it does require that drug effects be measured at time of steady state or peak effect. Based on previous studies in volunteers, we assumed that intrathecal neostigmine would have a constant analgesic effect from 60–180 min after injection, and that targeted epidural clonidine infusions would achieve a steady state analgesic effect within 60 min.[6,12] These assumptions were met, as shown by dose-dependent analgesia 60 min after intrathecal neostigmine injection and constant analgesia 60 and 120 min after beginning epidural clonidine infusions in those receiving epidural clonidine targeted to not change during that time.

Unlike studies in rats, the current study in humans demonstrated an interaction between neostigmine and clonidine that was clearly additive, not synergistic. Several explanations are possible. It is conceivable that, despite infusion of clonidine to targeted CSF concentrations, we may have missed a synergistic interaction by administering one drug intrathecally and the other epidurally. This would be especially likely if the large dose of clonidine necessary with epidural administration produced analgesia by systemic absorption and a nonspinal mechanism. Arguing against this possibility is the more profound analgesia obtained during acute clonidine administration epidurally than intravenously,[21,22] and greater analgesia in foot than hand after lumbar epidural infusion. It is also possible that other ratios of drug administration could yield a synergistic interaction. Neostigmine's side effects, especially nausea, precluded investigating larger doses. Rats with chronic intrathecal cannulas, which demonstrate a clonidine-neostigmine synergy,[2] may have been chronically stressed, which could enhance neostigmine-induced analgesia[7] and perhaps alter its interaction with clonidine. Finally, it is possible that the positive interaction between intraspinal neostigmine and clonidine in humans is additive in contrast to that in rats.

The current study provides several clues to the mechanisms of actions of these drugs. Hyperbaric neostigmine produces analgesia by a spinal mechanism, as demonstrated by analgesia when injected in subjects in the lateral position, but not in the sitting position. Clinical experience demonstrates that similarly restricted actions can be achieved by injection of hyperbaric solutions of local anesthetics in subjects in the sitting position. Both clonidine and neostigmine produced greater analgesia in the foot than in the hand after lumbar administration, supporting a spinal site of action. Interestingly, despite a lack of analgesia when injected in subjects in the sitting position, intrathecal neostigmine injection in each position yielded similar lumbar CSF concentrations of neostigmine and acetylcholine 2 h later. This may well reflect similar concentrations at a site (lower lumbar interspace) considerably caudad to neostigmine's site of action (the spinal cord). As previously observed, epidural clonidine increases acetylcholine in human CSF,[17] and a plateau is reached in the dose response to neostigmine in increasing CSF acetylcholine.[6]

Several observations in animals support a role for spinal nitric oxide synthesis in the analgesic actions of acetylcholine in the spinal cord. For example, antinociception from intrathecal injection of muscarinic agonists is blocked by nitric oxide synthase inhibitors,[23]

and enhancement of clonidine analgesia by neostigmine is blocked by nitric oxide synthase inhibitors.[7] Intrathecal clonidine antinociception is also enhanced by l-arginine, an effect blocked by nitric oxide synthase inhibitors,[24] supporting a role for nitric oxide in clonidine analgesia. Because nitric oxide stimulates guanylate cyclase, we tested a nitric oxide mechanism by measuring cGMP in CSF after intrathecal neostigmine and epidural clonidine. The lack of effect of these drugs in analgesic doses on CSF cGMP does not support such a nitric oxide mechanism, although many other explanations are conceivable, and this observation does not exclude this possibility.

Epidural clonidine reduces sympathetic nervous system activity and blood pressure by direct spinal as well as supraspinal actions,[25,26] whereas intrathecal neostigmine increases both in animals by spinal actions.[27,28] We did not observe increased blood pressure in this study, as in a previous one,[6] after intrathecal neostigmine injection. However, neostigmine increased plasma norepinephrine concentrations after injection in subjects in the lateral, but not the sitting, position, consistent with mild sympathetic stimulation. Similarly, intrathecal neostigmine injection inhibits hypotension from intrathecal clonidine in animals,[28] and inhibited clonidine-induced reductions in plasma norepinephrine and blood pressure when injected in subjects in the lateral, but not the sitting, position in this study in humans.

Nausea and subjective weakness represent important limitations to intrathecal neostigmine therapy, just as sedation does with epidural clonidine therapy. Despite the positive interaction between these agents for analgesia, they did not enhance each others' side effects. In addition, this study confirms mild to no respiratory depression from these drugs alone or in combination at profoundly analgesic doses. Therefore, this combination possesses many desirable characteristics for clinical application.

In summary, both intrathecal neostigmine and epidural clonidine produce analgesia, and interact additively in human volunteers subjected to a noxious cold stimulus. Both drugs produce more analgesia in the foot than in the hand after lumbar administration, and neostigmine produces more analgesia after lumbar injection of a hyperbaric solution in subjects in the lateral than in the sitting position, consistent with a spinal site of action. Neostigmine partially inhibits reduced blood pressure and plasma norepinephrine from epidural clonidine, but, otherwise, these drugs do not alter each

others' side effects or cause significantly respiratory depression. The eventual clinical utility of this combination deserves investigation.

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