Anesthesiology
78:301–307, 1993

Neostigmine Counteracts Spinal Clonidine-induced Hypotension in Sheep

Julie S. Williams, M.S., Chuanyao Tong, M.D.,† James C. Eisenach, M.D.‡*

Background: **Intraspinal clonidine injection produces analgesia free of respiratory depression, but also decreases blood pressure and causes sedation. Spinal neostigmine injection alone increases blood pressure in animals and enhances clonidine-induced analgesia.**

Methods: **To test whether neostigmine would alter clonidine-induced hypotension, nine chronically prepared sheep received intrathecal injections of saline or neostigmine (150, 300, 1,000 μg) followed in 15 min by 200 μg clonidine.**

Results: **Clonidine plus saline decreased mean arterial pressure by 12 ± 3% associated with small, statistically nonsignificant decreases in heart rate, cardiac output, and systemic vascular resistance. Prior injection of neostigmine diminished hypotension 60 min after clonidine injection in a dose-dependent manner. To further define the time course and pharmacology of this interaction, seven other sheep received intrathecal saline, neostigmine (1,000 μg), or neostigmine plus methylatropine (1,000 μg) 75 min prior to 200 μg clonidine. With this longer interval between injections, neostigmine abolished clonidine-induced hypotension, and this protective effect was inhibited by methylatropine. To test whether rostral spread of neostigmine in cerebrospinal fluid would alter its hemodynamic effects, we injected intrathecal neostigmine into the upper cervical site. Intrathecal neostigmine increased mean arterial pressure and heart rate at this site to a degree similar to that in the thoracic area, with no effect on behavioral or arterial blood gas tensions.**

Conclusions: **These data are consistent with neostigmine's counteraction of clonidine-induced hypotension by a spinal muscarinic mechanism and support investigation of spinal α_2-adrenergic-cholinergic combinations for pain therapy. (Key words: Acetylcholine. Anesthetic techniques: spinal. Cholinesterase inhibitors: neostigmine. Spinal cord: intermediolateral cell column. Sympathetic nervous system: α_2-adrenergic agonists; clonidine.)**

INTRASPINAL administration of α_2-adrenergic agonists holds the potential for providing excellent analgesia free of the risk of significant respiratory depression. However, initial experience with epidural and intrathecal administration of the α_2-adrenergic agonist clonidine also has demonstrated hypotension and dose-dependent sedation.[1,2] These side effects are well tolerated in normal individuals, but are bothersome and could be dangerous in patients with cardiovascular disease.

One approach to diminish the side effects from α_2-adrenergic agonists is to combine them with other analgesic agents, thereby decreasing the dose of α_2-adrenergic agonist required. For example, opioids and α_2-adrenergic agonists interact synergistically to produce analgesia in animals,[3] and epidural clonidine injection prolongs postoperative analgesia provided by fentanyl in humans.[4] Decreasing clonidine dose by combining it with opioid should diminish clonidine-induced sedation. However, because epidurally administered clonidine has a U-shaped dose response on blood pressure (BP),[5] decreased clonidine dose may not lessen hypotension. For this reason, addition of opioid to clonidine may exacerbate rather than lessen hypotension.

Recent description of α_2-adrenergic-cholinergic interactions in spinal sensory processing[6] suggests combinations of these agents may be more effective than α_2-adrenergic-opioid combinations in producing analgesia while minimizing side effects. This is because, unlike opioids, spinal cholinergic agonist injection increases spinal preganglionic sympathetic nervous system activity, thereby increasing BP and heart rate (HR).[7] We now report the hemodynamic interactions between spinally administered clonidine and neostigmine, and demonstrate the pharmacology and time course of neostigmine's inhibition of spinal clonidine-induced hy-

* Medical Student, Bowman Gray School of Medicine, Winston-Salem, North Carolina.

† Research Assistant Professor of Anesthesia, Wake Forest University Medical Center.

‡ Associate Professor of Anesthesia, Wake Forest University Medical Center.

Received from the Department of Anesthesia, Wake Forest University Medical Center, Winston-Salem, North Carolina. Accepted for publication October 2, 1992. Supported in part by grants GM35523 and T32-DK-07400 from the National Institutes of Health. Presented in part at the annual meeting of the American Society of Regional Anesthesia, Tampa, Florida, March 26–29, 1992.

Address reprint requests to Dr. Eisenach: Department of Anesthesia, Wake Forest University Medical Center, Medical Center Boulevard, Winston-Salem, North Carolina 27157-1009.

potension. Since the highly polar neostigmine may circulate in cerebrospinal fluid (CSF) to brainstem sites of cardiovascular control, we also examined the effects of upper cervical intrathecal injection of neostigmine.

Methods

Following approval by the Animal Care and Use Committee of the authors' institution, 21 ewes of mixed Western breeds were studied. After a 48-h fast, anesthesia was induced with intravenous ketamine (10 mg/kg) plus pentobarbital (15 mg/kg), the trachea intubated, and anesthesia maintained with 1–1.5% halothane in oxygen. Polyvinyl catheters were inserted under direct vision into a femoral artery and vein and advanced into the distal aorta and inferior vena cava, respectively. An 8.5-French introducer was inserted percutaneously into the right common jugular vein; the animal turned prone; and in 16 ewes, a hemilaminectomy performed at the lumbosacral interspace. A single distal port catheter (21-G, Portex™, Keene, NH) was inserted 28 cm cephalad through a small nick in the dura, such that its tip would be located at an upper thoracic dermatome. This site was chosen to maximize clonidine-induced hypotension in sheep. In five ewes, an intrathecal catheter was inserted with tip at C2–C3 *via* a cervical laminectomy. Catheters were secured and maintained in a canvas pouch on the flank and the animal allowed to awaken from anesthesia. All animals were standing, eating, and drinking normally within 3 h of surgery. Intravenous meperidine (75 mg) was to be administered postoperatively to treat behavioral signs of pain. In no cases were abnormal behaviors noted. Animals received 1 g intramuscular kanamycin for 2 days postoperatively. On the third postoperative day, a 7.5-French pulmonary artery catheter (American Edwards™, Irvine, CA) was inserted through the jugular venous introducer under pressure waveform guidance and secured in place.

Neostigmine Dose Response

On the day of the study, the femoral and pulmonary arterial catheters in nine ewes were connected with pressure transducers (Viggo-Spectromed™, Oxnard, CA) for continuous monitoring of systemic, right atrial, and pulmonary pressures and HR using a Grass™ (Quincy, MA) polygraph and on-line computer data acquisition system. These values were recorded at 1-min intervals and values obtained at the same times as cardiac output determinations used for calculation of vascular resistance. After 30 min of baseline measures, animals received, in a random order with experiments separated by at least 48 h, either saline or neostigmine (150, 300, 1,000 μg) followed in 15 min by 200 μg clonidine. Cardiac output was determined by thermodilution in triplicate with 5 ml iced 5% dextrose solution injection at baseline, just prior to clonidine injection and at 15, 30, and 60 min after clonidine injection.

Neostigmine Time Course and Pharmacology

In separate experiments, seven other ewes received 1,000 μg intrathecal saline or neostigmine, or 1,000 μg neostigmine plus methylatropine, followed in 75 min by intrathecal 200 μg clonidine. Measurements were obtained as described above at baseline, just prior to clonidine injection, and 15 min after clonidine injection.

Cervical Intrathecal Injection of Neostigmine

In a separate experiment, five ewes received 1,000 μg intrathecal neostigmine *via* a catheter with tip at C2–C3. Measurements were obtained as described above at baseline and at 15, 30, and 60 min after neostigmine injection. At these times arterial blood was sampled and analyzed for blood gas tensions and pH using a Radiometer™ microanalyzer.

Drugs and Solutions

Halothane, kanamycin, ketamine, and pentobarbital were obtained from Barber Veterinary Supply Co. (Richmond, VA). Methylatropine and neostigmine bromide were obtained from Sigma Chemical Co. (St. Louis, MO). Clonidine hydrochloride was a gift from Fujisawa Pharmaceutical Co. (Deerfield, IL). Drugs were dissolved in sterile saline, and intrathecal injections were administered in a volume of 0.5 ml followed by flush of 0.5 ml (two times catheter dead space) of sterile saline.

Statistics

Data are expressed as mean ± SEM. Although presented in some cases for clarity as percent change from baseline, all data analyses were performed on the raw data. Effect of drug treatment over time was assessed by one-way analysis of variance for repeated measures followed by Dunnett's test, and experimental groups were compared over the entire time range by two-way

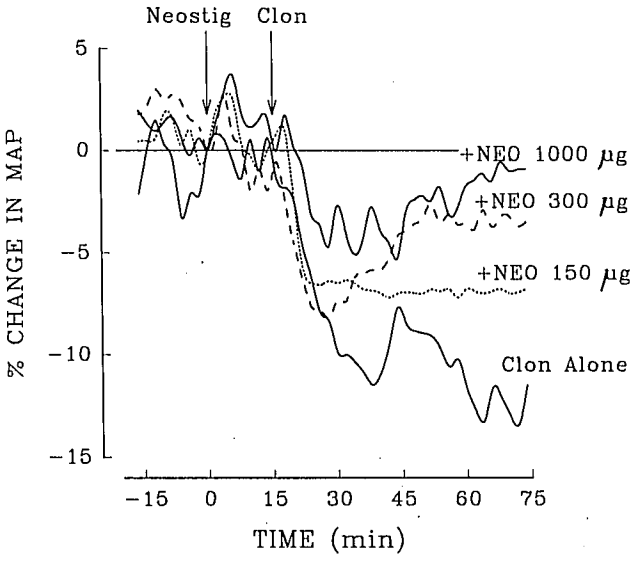

Fig. 1. Percentage change in mean arterial pressure over time in animals receiving clonidine along (lower solid line) or clonidine preceded by 15 min with 150 μg (dotted line), 300 μg (dashed line), or 1,000 μg (upper solid line) neostigmine. Time of neostigmine (or saline) and clonidine injection indicated by arrows. Pressure change is 0 at time of first injection. Each line represents the mean of 5–8 animals. For SEM, see table 1. Curves differ by two-way analysis of variance, with 1,000 μg neostigmine = 300 μg > 150 μg > saline.

analysis of variance for repeated measures and at each time by one-way analysis of variance for repeated measures. $P < .05$ was considered significant.

Results

15-min Pretreatment

Intrathecal clonidine after saline pretreatment significantly decreased mean arterial pressure (MAP; fig. 1). Decreased MAP after clonidine was associated with minor reductions in HR and cardiac output that did not in themselves achieve statistical significance (table 1). Clonidine-induced hypotension was unaffected by pretreatment with 150 μg neostigmine, but was reduced in magnitude by both 300 and 1,000 μg neostigmine (fig. 1). Examination of the time course of clonidine-induced hypotension revealed a nonsignificant effect of neostigmine 15 min after clonidine injection, but dose-dependent counteraction 60 min after clonidine injection (fig. 1). Neostigmine alone did not affect hemodynamic variables over the 15 min until clonidine was injected (table 1). However, neostigmine without clonidine yielded a prolonged increase in MAP, differing from the decrease in MAP produced by clo-

Table 1. Hemodynamic Parameters: Injections Separated by 15 Min

Group*	Time (min)	Mean Arterial Pressure (mmHg)	Heart Rate (beats/min)	Cardiac Output (L/min)	System Vascular Resistance (mmHg · min^{-1} · L^{-1})
Clonidine alone	−15	112 ± 2	123 ± 11	7.7 ± 0.6	14 ± 1.2
	0	113 ± 2	121 ± 12	7.7 ± 0.6	14 ± 1.2
	15	102 ± 4†	123 ± 12	6.7 ± 0.4	14 ± 1.4
	30	99 ± 3†	119 ± 10	6.5 ± 0.5	14 ± 1.3
	60	99 ± 3†	113 ± 11	6.7 ± 0.3	13 ± 0.4
Clonidine + neostigmine, 150 μg	−15	109 ± 5	119 ± 11	6.1 ± 0.4	17 ± 0.9
	0	106 ± 4	123 ± 11	6.6 ± 0.4	15 ± 1.0
	15	101 ± 3†	114 ± 7	6.4 ± 0.6	15 ± 1.5
	30	101 ± 3†	124 ± 13	6.3 ± 0.5	15 ± 1.5
	60	101 ± 3†	121 ± 13	6.2 ± 0.4	15 ± 1.5
Clonidine alone + neostigmine, 300 μg	−15	98 ± 5	120 ± 3	7.2 ± 0.7	13 ± 1.6
	0	94 ± 6	119 ± 4	7.6 ± 0.6	11 ± 1.3
	15	93 ± 5†	113 ± 6	6.8 ± 0.4	12 ± 0.6
	30	93 ± 8	112 ± 5	6.6 ± 0.7	13 ± 1.6
	60	95 ± 5	111 ± 5	6.8 ± 0.6	13 ± 1.4
Clonidine alone + neostigmine, 1,000 μg	−15	101 ± 8	117 ± 9	6.8 ± 0.6	15 ± 1.9
	0	101 ± 7	125 ± 11	7.3 ± 0.3	13 ± 1.3
	15	96 ± 7	115 ± 13	6.0 ± 0.4	15 ± 1.5
	30	96 ± 7	117 ± 12	6.2 ± 0.6	15 ± 1.7
	60	99 ± 6	123 ± 12	6.6 ± 0.8	14 ± 1.5

* Clonidine, 200 μg, injected in all groups at time 0; saline or neostigmine injected at time −15.

† $P < .05$ *versus* baseline (−15 min) observation.

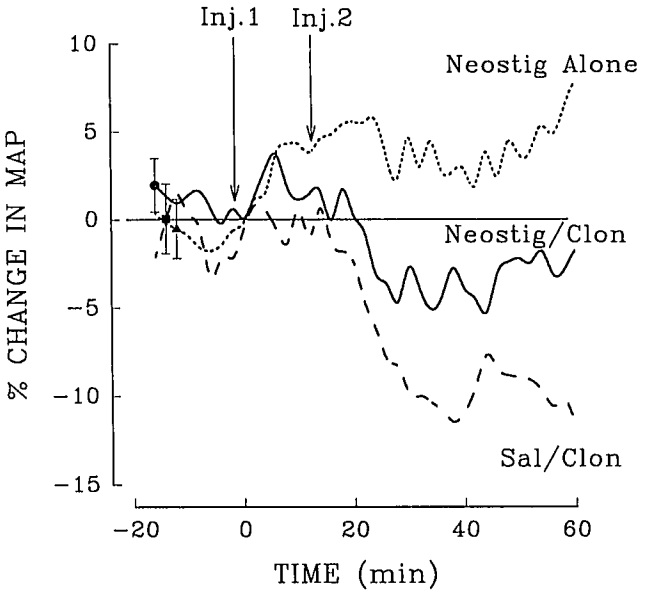

Fig. 2. Percentage change in mean arterial pressure after injection of 1,000 μg neostigmine at time 0 (dotted line), saline at time 0 and clonidine at time 15 min (dashed line), and 1,000 μg neostigmine at time 0 and clonidine at time 15 min (solid line). Time of injections indicated by arrows. Pressure change is 0 at time of first injection. Each line represents the mean of 5–7 animals. Examples of individual data points' SEM shown for neostigmine/clonidine (●), saline/clonidine (■), and neostigmine alone (▲). All three curves curves differ by two-way analysis of variance ($P < .05$).

nidine alone and the lack of effect on MAP produced by their combination (fig. 2).

75-min Pretreatment

As in the other injection regimen, intrathecal clonidine after saline pretreatment significantly decreased MAP (fig. 3), and this decrease was associated with small changes in HR, cardiac output, and systemic vascular resistance that were not in themselves significant (table 2). Neostigmine (1,000 μg) increased MAP 75 min after injection, and counteracted hypotension 15 min after clonidine injection (fig. 3). Combination of methylatropine with neostigmine diminished neostigmine's protection against clonidine-induced hypotension (fig. 3, table 2).

Cervical Injection

Cervical intrathecal injection of neostigmine increased MAP and HR with a time course similar to that of thoracic intrathecal injection (fig. 4, table 3). In addition, no abnormal behaviors were noted in sheep after cervical intrathecal neostigmine injection, and arterial blood gas tensions and pH were unaffected by neostigmine (table 3).

Discussion

Previous studies have demonstrated that intraspinal injection of cholinergic agonists or cholinesterase inhibitors increases BP and HR,[7–9] whereas injection of α_2-adrenergic agonists decreases BP and HR.[10,11] The current study, coupled with neurophysiologic and functional studies demonstrating enhancement of analgesia by combination of these agents,[6] provides the rationale for clinical development of such combination therapy.

Site of Action

Work in this and other laboratories suggest both clonidine and neostigmine affect BP and HR after intrathecal injection by actions within the spinal cord. For example, there is a dense binding of α_2-adrenergic ligands in the intermediolateral cell column,[12] and iontophoretic application of clonidine at this site or by intrathecal injection decreases sympathetic neural activity.[13] Clonidine decreases BP more after spinal thoracic than

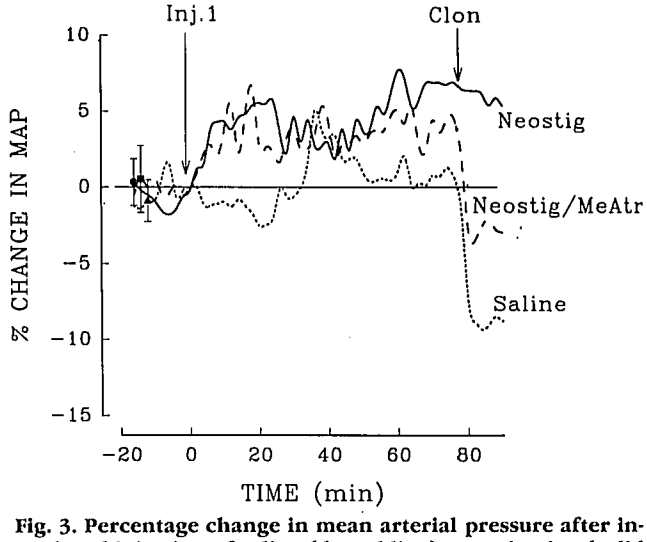

Fig. 3. Percentage change in mean arterial pressure after intrathecal injection of saline (dotted line), neostigmine (solid line), or neostigmine plus methylatropine (dashed line) at time 0, and intrathecal injection of clonidine at time 75 min. Pressure change is 0 at the time of first injection. Each line represents the mean of 5–7 animals. Examples of individual data points' SEM shown for saline (▲), neostigmine (●), and neostigmine/atropine (■). Neostigmine curve differs from saline and neostigmine/methylatropine curves by two-way analysis of variance.

Table 2. Hemodynamic Parameters: Injections Separated by 75 Min

Group*	Time (min)	Mean Arterial Pressure (mmHg)	Heart Rate (beats/min)	Cardiac Output (L/min)	System Vascular Resistance (mmHg · min^{-1} · L^{-1})
Clonidine alone	0	100 ± 6	114 ± 6	6.6 ± 0.6	15 ± 0.9
	30	110 ± 6	114 ± 5	—	—
	60	101 ± 6	111 ± 5	6.7 ± 0.6	15 ± 0.7
	75	101 ± 7	115 ± 5	6.3 ± 0.6	16 ± 2.2
	90	91 ± 4†	104 ± 3†	6.5 ± 0.6	14 ± 2.0
Clonidine + neostigmine, 1,000 μg	0	91 ± 3	105 ± 4	6.3 ± 0.8	14 ± 1.2
	30	95 ± 4	108 ± 5	—	—
	60	98 ± 3†	108 ± 5	6.4 ± 0.7	15 ± 1.5
	75	97 ± 3†	117 ± 7†	6.8 ± 0.7	15 ± 1.7
	90	96 ± 4	111 ± 8	6.4 ± 0.4	14 ± 0.9
Clonidine alone + neostigmine, 1,000 μg, + methylatropine, 1,000 μg	0	91 ± 4	108 ± 4	6.3 ± 0.9	14 ± 1.8
	30	94 ± 4	121 ± 10	—	—
	60	96 ± 4	108 ± 4	6.4 ± 0.6	14 ± 1.7
	75	95 ± 2	115 ± 7	6.0 ± 0.3	15 ± 1.0
	90	91 ± 3	104 ± 3	5.0 ± 0.5	16 ± 1.8

* First injection (saline, neostigmine, or neostigmine + methylatropine) at time 0; clonidine, 200 μg, at time 75.

† $P < .05$ *versus* baseline.

lumbar or cervical injection in sheep[10] and humans,[14] in accordance with anatomic location of preganglionic sympathetic neurons.

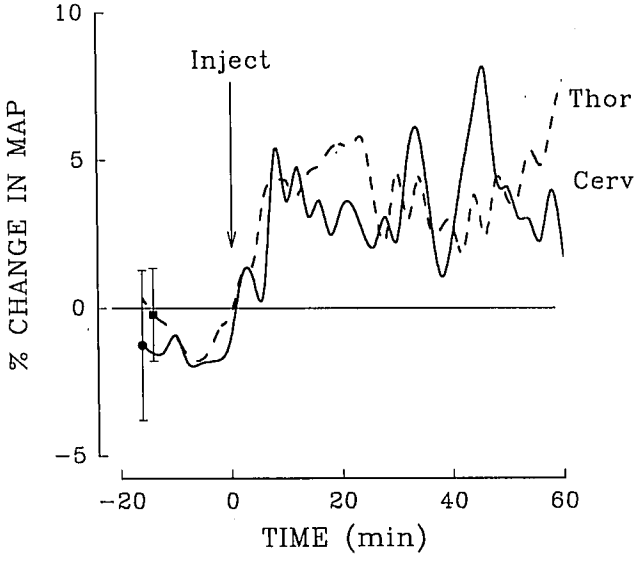

Fig. 4. Percentage change in mean arterial pressure after cervical (solid lines) or thoracic (dashed line) intrathecal injection of 1,000 μg neostigmine at time 0. Pressure change is 0 at time of first injection. Each line represents the mean of 5–7 animals. Examples of individual data points' SEM shown for cervical (●) and thoracic (■) neostigmine. Curves do not differ by two-way analysis of variance.

In contrast to this α_2-adrenergic mechanism, cholinergic systems excite spinal preganglionic sympathetic neurons. There is a dense binding of cholinergic ligands within the intermediolateral cell column,[12] and iontophoretic application of the muscarinic agonist carbachol at this site[15,16] or by intrathecal injection[7–9] increases BP and HR. Whether this is due to local actions within the cord or to activation of ascending pressor systems is unclear.[7] Nonetheless, the current study agrees with results of work in rodents[7] and sheep[10] to demonstrate increased BP and HR by cholinergic stimulation at a spinal site, and agrees that such cholinergic systems must be tonically active, since BP and HR are increased by local cholinesterase inhibition. Based on our results, we propose that spinal injection of neostigmine directly counterbalances clonidine's effects in the intermediolateral cell column, thereby eliminating clonidine-induced hypotension and bradycardia.

Rostral spread of drugs by circulation in CSF can produce effects by actions in the brainstem, such as delayed respiratory depression after spinally administered morphine. Clonidine could produce delayed hypotension by action at brainstem sites. Such delayed hypotension has not been observed in animals or humans,[1,2] perhaps due to clonidine's high lipid solubility, limiting its residence time in CSF. Neostigmine, however, is much less lipid soluble, and could increase MAP by actions at cholinergic sites in the brainstem. The present study,

Table 3. Effects of Cervical Intrathecal Neostigmine on Hemodynamic Parameters and Arterial Blood Gas Tensions and *p*H

Time (min)	Mean Arterial Pressure (mmHg)	Heart Rate (beats/min)	Cardaic Output (L/min)	Systemic Vascular Resistance ($mmHg \cdot min^{-1} \cdot L^{-1}$)	P_{O_2} (mmHg)	P_{CO_2} (mmHg)	*p*H
Baseline	93 ± 3	119 ± 4	6.6 ± 0.7	14 ± 1.7	111 ± 8	34 ± 1.0	7.45 ± 0.03
15	99 ± 4	134 ± 11	7.6 ± 0.7	12 ± 1.3	119 ± 5	30 ± 2.8	7.48 ± 0.04
30	100 ± 2*	144 ± 8*	8.0 ± 0.6	12 ± 1.3	119 ± 7	28 ± 4.7	7.52 ± 0.07
60	99 ± 3*	138 ± 11*	8.0 ± 0.9	12 ± 1.5	117 ± 4	27 ± 3.6	7.52 ± 0.05
120	97 ± 2	138 ± 11*	7.4 ± 0.4	12 ± 0.9	116 ± 4	31 ± 2.8	7.47 ± 0.04

Neostigmine was injected as time 0.

* $P < .05$ *versus* baseline.

however, observed only small increases in MAP by upper cervical intrathecal injection of neostigmine, without behavioral evidence of cholinergic crisis or effects on respiration.

Receptor Subtypes

Clonidine acts in the spinal cord to decrease sympathetic nervous outflow by local actions on α_2-adrenergic receptors, as evidenced by reversal with α_2-adrenergic antagonist.[10] There are no current anatomic, neurophysiologic, or functional data defining which of the multiple α_2-adrenoceptor subtypes are responsible for this effect. Similarly, cholinergic receptor subtypes involved in spinal control of sympathetic nervous system activity are not known. Reversal of neostigmine's effect by methylatropine in the current study is in agreement with previous work demonstrating that cholinergic actions on hemodynamic sites in the spinal cord are due to muscarinic, not nicotinic, receptors.[16] Functional studies suggest that this may be due to actions on the M_2 muscarinic receptor subtype,[16] although neurophysiologic studies with receptor subtype-selective agents have not been conducted.

Time Course of Drug Effects

Spinal effects of drugs following intrathecal injection correlate closely with time of penetration by diffusion of these drugs to their active sites in the cord.[17,18] Clonidine is highly lipid soluble and may diffuse rapidly from CSF to hemodynamic sites in the intermediolateral cell column, as witnessed by rapid onset of decreased BP and HR following spinal injection in animals and humans. In contrast, neostigmine is poorly lipid soluble, explaining absence of central effects after intravenous injection and delayed inhibition of clonidine-induced hemodynamic depression in the current study.

Whereas differences in lipid solubility offer a likely explanation for the time course of effects in this study, their potential clinical relevance is less clear. For example, clonidine-induced analgesia is relatively brief after bolus epidural injection, necessitating continuous infusion for sustained analgesia. Addition of a more lipid soluble cholinesterase inhibitor, such as physostigmine, to clonidine may protect immediately from cardiovascular depression. However, protection against clonidine-induced hypotension during continuous infusion may require relatively large doses of physostigmine by continuous infusion, which could produce significant peripheral side effects. It may be that pretreatment with a single dose of spinal neostigmine would be more advantageous, due to neostigmine's long half-life in CSF after spinal injection, and restriction to CSF (unpublished observations).

Analgesic Interactions

Both muscarinic and α_2-adrenergic receptors are involved in sensory processing in the spinal cord. There is dense binding of specific radioligands to each of these receptor types in the superficial layers of the spinal cord,[19] and spinal injection of direct agonists for these receptors produces behavioral analgesia in animals.[20] Intraspinal clonidine injection produces analgesia in humans,[1,2] and although the analgesic effect of intraspinal injection of cholinesterase inhibitors has not been examined, intravenous injection of lipid soluble cholinesterase inhibitors produces analgesia in humans.[21]

Studies in this laboratory and by Gordh *et al.*[6] are consistent with the hypothesis that spinal α_2-adrenergic agonists produce analgesia in large part by causing acetylcholine release. As such, intraspinal clonidine increases CSF concentration of acetylcholine in sheep[22] and humans (unpublished observations) by an α_2-adrenergic mechanism, and intrathecal clonidine anal-

gesia is inhibited by atropine and enhanced by neostigmine.[6]

In summary, intrathecal clonidine injection in conscious sheep decreases BP, an effect counteracted by pretreatment with spinal neostigmine. Neostigmine given 15 min before clonidine counteracted clonidine-induced hypotension 60 min, but not 15 min after clonidine injection. Neostigmine given 75 min before clonidine counteracted clonidine-induced hemodynamic depression, and neostigmine's effect was abolished by spinal methylatropine. These results can be explained by differences in lipid solubility between these agents and opposing spinal actions on muscarinic and α_2-adrenergic receptors involved in regulating sympathetic outflow. Upper cervical intrathecal injection of neostigmine also increases MAP, likely due to actions in the brainstem, without effect on behavior or arterial blood gas tensions. Neostigmine has not been investigated adequately for safety by intrathecal injection, precluding clinical use.[23] However, these data, coupled with observations of enhancement of α_2-adrenergic analgesia by cholinesterase inhibitors, provide the rationale for further preclinical investigation of this drug combination.

References

1. Bonnet F, Boico O, Rostaing S, Saada M, Loriferne J-F, Touboul C, Abhay K, Ghignone M: Postoperative analgesia with extradural clonidine. Br J Anaesth 63:465–469, 1989

2. Eisenach JC, Lysak SZ, Viscomi CM: Epidural clonidine analgesia following surgery: Phase I. ANESTHESIOLOGY 71:640–646, 1989

3. Ossipov MH, Harris S, Lloyd P, Messineo E: An isobolographic analysis of the antinociceptive effect of systemically and intrathecally administered combinations of clonidine and opiates. J Pharmacol Exp Ther 255:1107–1116, 1990

4. Rostaing S, Bonnet F, Levron JC, Vodinh J, Pluskwa F, Saada M: Effect of epidural clonidine on analgesia and pharmacokinetics of epidural fentanyl in postoperative patients. ANESTHESIOLOGY 75:420–425, 1991

5. Eisenach JC, Dewan DM: Intrathecal clonidine in obstetrics: Sheep studies. ANESTHESIOLOGY 72:663–668, 1990

6. Gordh T Jr, Jansson I, Hartvig P, Gillberg PG, Post C: Interactions between noradrenergic and cholinergic mechanisms involved in spinal nociceptive processing. Acta Anaesthesiol Scand 33:39–47, 1989

7. Buccafusco JJ, Magri' V: The pressor response to spinal cholinergic stimulation in spontaneously hypertensive rats. Brain Res Bull 25:69–74, 1990

8. Takahashi H, Buccafusco JJ: Spinal cholinergic modulation of cardiovascular tone and somatosympathetic reflex response. Brain Res Bull 27:47–51, 1991

9. Takahashi H, Buccafusco JJ: The sympathoexcitatory response following selective activation of a spinal cholinergic system in anesthetized rats. J Auton Nerv Syst 34:59–68, 1991

10. Eisenach JC, Tong C: Site of hemodynamic effects of intrathecal α_2-adrenergic agonists. ANESTHESIOLOGY 74:766–771, 1991

11. Nagasaka H, Yaksh TL: Pharmacology of intrathecal adrenergic agonists: Cardiovascular and nociceptive reflexes in halothane-anesthetized rats. ANESTHESIOLOGY 73:1198–1207, 1990

12. Seybold VS, Elde RP: Receptor autoradiography in thoracic spinal cord: Correlation of neurotransmitter binding sites with sympathoadrenal neurons. J Neurosci 4:2533–2542, 1984

13. Guyenet PG, Cabot JB: Inhibition of sympathetic preganglionic neurons by catecholamines and clonidine: Mediation by an α-adrenergic receptor. J Neurosci 1:908–917, 1981

14. De Kock M: Site of hemodynamic effects of α_2-adrenergic agonists. ANESTHESIOLOGY 75:715–716, 1991

15. Sundaram K, Murugaian J, Krieger A, Sapru H: Microinjections of cholinergic agonists into the intermediolateral cell column of the spinal cord at T_1–T_3 increase heart rate and contractility. Brain Res 503:22–31, 1989

16. Calaresu FR, McKitrick DJ, Weernink EJ: Microinjection of substance P and ACh into rat intermediolateral nucleus elicits cardiovascular responses. Am J Physiol 259:R357–R361, 1990

17. Post C, Gordh T Jr, Minor BG, Archer T, Freedman J: Antinociceptive effects and spinal cord tissue concentrations after intrathecal injection of guanfacine or clonidine into rats. Anesth Analg 66:317–324, 1987

18. Gustafsson LL, Post C: The degree of analgesia correlates to spinal morphine concentrations after intrathecal administration in rats. Acta Pharmacol Toxicol 58:243–248, 1986

19. Seybold VS: Distribution of histaminergic, muscarinic and serotonergic binding sites in cat spinal cord with emphasis on the region surrounding the central canal. Brain Res 342:291–296, 1985

20. Zhuo M, Gebhart GF: Tonic cholinergic inhibition of spinal mechanical transmission. Pain 46:211–222, 1991

21. Petersson J, Gordh TE, Hartvig P, Wiklund L: A double-blind trial of the analgesic properties of physostigmine in postoperative patients. Acta Anaesthesiol Scand 30:283–288, 1986

22. Tong C, Eisenach JC, Shea P, Bucklin B, Limauro D: Does intrathecal clonidine release acetylcholine (abstr)? ANESTHESIOLOGY 75: A658, 1991

23. Yaksh TL, Collins JG: Studies in animals should precede human use of spinally administered drugs (editorial). ANESTHESIOLOGY 70: 4–6, 1989