Cardiorespiratory and Spinal Cord Blood Flow
Effects of Intrathecal Neostigmine Methylsulfate, Clonidine, and Their Combination in Sheep

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Background: Intrathecal neostigmine may produce analgesia by itself and may enhance analgesia from spinal clonidine. Before clinical trials, the spinal cord blood flow effects of these drugs alone and in combination should be examined in animals.

Methods: Conscious, nonpregnant ewes with indwelling vascular and thoracic spinal catheters received intrathecal injection of 0.2 or 2 mg neostigmine, 0.2 mg clonidine, or 2 mg neostigmine plus 0.2 mg clonidine. Mean systemic and pulmonary arterial and central venous pressures, heart rate, and cardiac output were monitored. Arterial blood was sampled for blood gas tensions and pH, and spinal cord blood flow was determined by colored microsphere injection before and at 15, 60, and 240 min after spinal study drug injection.

Results: Neostigmine alone did not affect cardiorespiratory variables or spinal cord blood flow. Intrathecal clonidine alone decreased systemic arterial and central venous pressures, whereas these effects were not observed with addition of neostigmine. Clonidine or neostigmine alone or the combination of clonidine and neostigmine did not affect spinal cord blood flow.

Conclusions: Intrathecal neostigmine alone or in combination with clonidine does not reduce spinal cord blood flow.

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an important preclinical toxicity issue. These results provide additional support for initial clinical trials of intrathecal neostigmine for analgesia. (Key words: Acetylcholinesterase inhibitor: neostigmine. Anesthesia, spinal. Microspheres, colored. Spinal cord: blood supply; drug effects. Sympathetic nervous system, α receptor agonists: clonidine.)

THERE is a high density of muscarinic cholinergic receptors in the spinal cord dorsal horn,¹ and intrathecal injection of cholinergic agonists in animals causes behavioral analgesia by a muscarinic mechanism.² Intrathecally administered muscarinic agonists also cause motor dysfunction due to actions in the ventral horn,² and this effect has limited interest in clinical development of muscarinic agonists. Intrathecal injection of cholinesterase inhibitors, such as neostigmine, also causes analgesia and, at high doses, motor dysfunction, presumably by inhibited breakdown of acetylcholine in the spinal cord.³

We are exploring a new rationale for the clinical development of neostigmine as a spinal analgesic. Gورد et al.⁴ observed an enhancement of intrathecal clonidine analgesia by neostigmine in rats and postulated that intrathecally administered α₂-adrenergic agonists cause analgesia, in part by activating spinal cholinergic neurons. The interaction between intrathecal α₂-adrenergic agonists and neostigmine is synergistic.⁴ In addition, intrathecally administered muscarinic agonists or neostigmine increase blood pressure and heart rate by actions at the preganglionic sympathetic neurons,⁵,⁶ and addition of neostigmine to clonidine counteracts clonidine-induced hypotension in sheep.⁷ It is possible, therefore, that addition of neostigmine to intrathecally administered α₂-adrenergic agonists in humans may enhance analgesia while counteracting hypotension.

Before clinical introduction of a new drug for intrathecal administration, a variety of preclinical toxicologic assessments are necessary,⁸-¹¹ including effects
INTRATHecal NEOSTIGMINE AND SPINAL CORD BLOOD FLOW

on spinal cord blood flow. Although topically applied acetylcholine can dilate cerebral blood vessels in vitro and in vivo by an endothelium-dependent mechanism, the effects of intrathecally administered muscarinic agonists or cholinesterase inhibitors on spinal cord blood flow have not been examined. Clonidine alone does not affect spinal cord blood flow in sheep, although the effects of adding neostigmine to clonidine on this variable have not been determined. For these reasons, the purpose of this study was to determine the effects of intrathecally administered neostigmine alone and in combination with clonidine on cardiovascular variables, behavior, and spinal cord blood flow in conscious sheep.

Methods

After approval by the Animal Care and Use Committee, 17 ewes of mixed Western breeds were studied. Animals were fasted for 48 h, anesthesia was induced with a mixture of ketamine (3–15 mg/kg) plus pentobarbital (3–15 mg/kg), the trachea was intubated, and anesthesia was maintained with 1–1.5% halothane in oxygen via controlled ventilation. An 8.5-Fr introducer was inserted percutaneously into the right common jugular vein, and polyvinyl catheters were inserted under direct vision into a femoral artery and vein and advanced 15 cm centrally. A 7-Fr pigtail catheter was inserted under direct vision into an internal carotid artery and advanced under pressure guidance into the left ventricle. The animals were turned prone, and a low lumbar hemilaminotomy was performed. A single distal port catheter (Portex, Keen, NH) was inserted into the intrathecal space through a nick in the dura, and the catheter was advanced 5 cm cephalad. Lower thoracic intrathecal catheter tip position was verified at autopsy. All catheters were secured and placed into a canvas pouch on the flank, and the animal was allowed to awaken from anesthesia. All animals were standing, eating, and drinking normally within 3 h of surgery. Intravenous flunixin (1.1 mg/kg) was available postoperatively for behavioral signs of pain. In no case was pain behavior noted postoperatively. Animals received 1 g intramuscular kanamycin each day for 2 days postoperatively. On the third postoperative day, a 7.5-Fr pulmonary artery catheter (American Edwards, Irvine, CA) was inserted through the jugular venous introducer under pressure waveform guidance into the distal pulmonary artery and secured in place after verifying pulmonary capillary wedge tracings were obtained.

The protocol was divided into two phases. In the first phase, we administered 0.2 mg neostigmine (low dose) or 2.0 mg neostigmine (high dose) through the indwelling lumbar spinal catheter. Each of the seven ewes studied in this phase received a computer-generated random scheme of either the low- or high-dose neostigmine, followed by the remaining neostigmine dose after a 48-h recovery period. In the second phase of the protocol, each of ten different ewes received in random order either 0.2 mg clonidine or 0.2 mg clonidine plus 2.0 mg neostigmine, followed after 48 h by the remaining intrathecal clonidine drug regimen.

Experimental Protocol

On the day of the experiment, each ewe was placed into a portable metabolic cage in a quiet room. Femoral and pulmonary arterial catheters were connected to pressure transducers (Vigo-Spectromed, Oxnard, CA) for continuous monitoring of systemic, pulmonary, and central venous pressures 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 time as cardiac output determinations used for calculation of vascular resistance. The left ventricular catheter was connected to a pressure transducer to verify location within the left ventricle before beginning the experiment. After 60 min of baseline recordings, colored microspheres were injected through the left ventricular catheter. The spinal injection was performed, followed in 15, 60, and 240 min by left ventricular injection of microspheres, each time with a different color of microsphere. In addition, at each time of microsphere injection, arterial blood samples were obtained from blood gas tension and pH analysis using a Radiometer (Copenhagen, DK) microanalyzer, and cardiac output was determined by thermodilution in triplicate with injection of 5 ml iced 5% dextrose solution. Approximately 2–4 h after each experiment, the animals were observed for possible behavioral changes, such as refusal to eat or to continue standing in the metabolic cart, signs of agitation on approach by the animal technician, or signs of any other unusual behavior. In addition, the status of the animal was routinely verified and noted in writing each morning.

Spinal Cord Blood Flow

To determine spinal cord blood flow, 10 × 10⁷ presonicated colored microspheres (15 μm in diameter, Ultrasound, West Los Angeles, CA) were injected...
through the left ventricular catheter at each period. A unique color was used for each period. Each ewe, therefore, received up to $80 \times 10^7$ microspheres (four periods, two experiments). Immediately before microsphere injection, reference sampling from the femoral artery was begun at a rate of 9 ml/min$^{-1}$ and collected for a period of 2 min.

After completion of both spinal injection studies, each ewe was killed by induction of deep anesthesia with 50 mg/kg intravenous pentobarbital, followed by intravenous injection of saturated KCl solution (2 ml/kg) to produce cardiac standstill, which was verified by arterial pressure monitoring. A dorsal laminectomy was performed; and after visual verification of lower thoracic intrathecal catheter tip location, sections of spinal cord were removed. One 6-cm section surrounding the catheter tip (approximately T12-L2) was collected, and another section distant from the catheter tip (C5-C8) was collected as a control. A sample from each renal cortex was taken for blood flow determination to document adequate mixing of the microspheres in blood.

**Tissue Processing**

Tissue and reference blood samples were processed by EZ Trac Inc. (West Los Angeles, CA) and the microspheres counted by a previously described method. Briefly, each tissue sample was digested by alkaline hydrolysis in a heated solution until all of the tissue was homogenized into suspension. The homogenized solution was centrifuged and the supernatant aspirated leaving a small pellet containing the microspheres. The microsphere pellet was resuspended in MICROSPHERE counting reagent (EZ-Trac) and sonicated for even microsphere suspension, and the microspheres in solution were counted using the TRACKER 1000 (EZ-Trac). The TRACKER 1000 is an integrated microscopic workstation using a 80486 computer subsystem, true color digital image acquisition, and software allowing online computation of regional blood flow. Spinal cord and renal blood flow were determined by $Q_t = \frac{100 \times Q_r \times C_r}{C_t}$, where $Q_t$ = tissue blood flow (ml/min), $Q_r$ = reference sample blood flow (ml/min), and $C_t$ and $C_r$ = number of colored microspheres in the tissue and reference samples, respectively. Blood flow is represented in ml·min$^{-1}·100$ g$^{-1}$ tissue.

**Drugs and Solutions**

Halothane, kanamycin, ketamine, and pentobarbital were obtained from Barber Veterinary Supply Company (Richmond, VA). Neostigmine bromide was obtained from International Medical Systems, Ltd. (El Monte, CA). Clonidine hydrochloride was obtained from Sigma Chemical Company (St. Louis, MO). Drugs for intrathecal injection were dissolved in sterile saline, and intrathecal injections were administered in a volume of 2.5 ml, followed by a flush of 0.5 ml (two times the catheter deadspace) of sterile saline.

**Statistics**

Data are presented as mean ± SEM. Baseline values were compared among treatment groups by one-way analysis of variance (ANOVA), with $P < 0.05$ considered significant. A mixed model analysis for repeated measures was used to compare (1) low- and high-dose neostigmine groups, (2) clonidine alone and clonidine plus neostigmine groups, and (3) within each group, changes compared to baseline. A compound symmetry variance structure best fit the data and was used in the analysis. A mixed model analysis uses a maximum likelihood estimate instead of the least-squares method used by a traditional multivariate ANOVA with repeated measures. The mixed model analysis is particularly appropriate when there are missing observations within an experiment because all the data can be used for analysis. In contrast, multivariate ANOVA discards complete experiments if any observations are missing. Therefore, using a mixed model analysis allowed for a more robust analysis of the data in this study. For each variable, an experiment-wide significance level of 0.05 was used, with Bonferroni corrections for multiple comparisons.

**Results**

**Hemodynamic Effects**

Low- and high-dose neostigmine groups did not differ in baseline variables (table 1), except cardiac output, which was greater before high-dose than before low-dose neostigmine injection ($P < 0.01$). Statistical analysis revealed no differences between neostigmine dose groups in cardiorespiratory variables after injection. Within each group, there was no effect of either dose of neostigmine over time (table 1). The increase in heart rate 4 h after high-dose neostigmine was not significant after correction for multiple comparisons (corrected $P = 0.37$).

Clonidine-treated groups did not differ in baseline variables (table 2), except for cardiac output and arterial $P_{CO_2}$, which were lower, and arterial $P_{O_2}$, which
was greater before injection of clonidine alone than before injection of clonidine plus neostigmine ($P < 0.02$). Within the clonidine-alone group, spinal injection decreased systemic arterial and right atrial pressures compared to baseline (table 2). In contrast, combination of clonidine and neostigmine did not affect these variables (table 2). In comparing groups, clonidine alone differed from clonidine plus neostigmine in heart rate and right atrial pressure but not systemic arterial pressure after spinal injection, with larger decreases in heart rate and right atrial pressure from clonidine alone than from clonidine plus neostigmine ($P < 0.001$).

**Regional Blood Flow**

Groups did not differ in baseline blood flow of the thoracolumbar spinal cord segment surrounding the intrathecal catheter tip (fig. 1). Neither dose of neostigmine significantly altered spinal cord blood flow near the catheter tip (fig. 1). Similarly, clonidine, either alone or with neostigmine, had no effect on spinal cord blood flow near the catheter tip (fig. 1). No treatment affected blood flow in the cervical spinal cord far from the catheter tip (table 3). Renal cortical blood flows were equal in the right and left kidneys, were similar at baseline, and were unaffected by intrathecal drug injection (table 3).

**Behavioral Effects**

Neither neostigmine or clonidine alone nor their combination caused behavioral effects. In no case was there evidence of motor weakness or dysfunction by observation.

**Discussion**

An assessment of spinal cord blood flow is a vital element in preclinical toxicologic screening of a new agent for spinal use. This study provides unique data concerning the effects of intrathecally administered neostigmine on local spinal cord blood flow and confirms an important interaction between neostigmine and clonidine on cardiovascular regulation.
Table 2. Hemodynamic and Respiratory Variables Following Intrathecal Clonidine Alone and with Neostigmine

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>+15 min</th>
<th>+60 min</th>
<th>+4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonidine, 0.2 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)††</td>
<td>101 ± 7</td>
<td>93 ± 6</td>
<td>81 ± 6</td>
<td>105 ± 9</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>7.4 ± 0.4‡</td>
<td>6.3 ± 0.3</td>
<td>5.8 ± 0.6</td>
<td>7.5 ± 0.4</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)*</td>
<td>97 ± 4</td>
<td>86 ± 3§</td>
<td>85 ± 3</td>
<td>96 ± 4</td>
</tr>
<tr>
<td>Right atrial pressure (mmHg)*†</td>
<td>8 ± 2</td>
<td>7 ± 2</td>
<td>5 ± 1</td>
<td>4 ± 2§</td>
</tr>
<tr>
<td>Systemic vascular resistance (mmHg · L⁻¹)</td>
<td>12.7 ± 0.7</td>
<td>13.6 ± 1.3</td>
<td>14.6 ± 1.2</td>
<td>12.6 ± 0.8</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure (mmHg)</td>
<td>22 ± 4</td>
<td>19 ± 2</td>
<td>17 ± 1</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.46 ± 0.01</td>
<td>7.46 ± 0.01</td>
<td>7.49 ± 0.01</td>
<td>7.48 ± 0.01</td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td>29 ± 2†</td>
<td>35 ± 2</td>
<td>35 ± 2</td>
<td>32 ± 1</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>113 ± 4‡</td>
<td>101 ± 4</td>
<td>99 ± 4</td>
<td>103 ± 4</td>
</tr>
<tr>
<td>Clonidine, 0.2 mg + Neostigmine, 2 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)††</td>
<td>106 ± 9</td>
<td>104 ± 10</td>
<td>95 ± 8</td>
<td>127 ± 16</td>
</tr>
<tr>
<td>Cardiac output (L/min)</td>
<td>8.7 ± 0.9‡</td>
<td>7.4 ± 1.0</td>
<td>7.5 ± 0.6</td>
<td>9.0 ± 1.2</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>99 ± 4</td>
<td>86 ± 6</td>
<td>90 ± 5</td>
<td>97 ± 5</td>
</tr>
<tr>
<td>Right atrial pressure (mmHg)††</td>
<td>5 ± 1</td>
<td>5 ± 1</td>
<td>5 ± 2</td>
<td>4 ± 2†</td>
</tr>
<tr>
<td>Systemic vascular resistance (mmHg · L⁻¹)</td>
<td>12.0 ± 1.3</td>
<td>12.9 ± 2.2</td>
<td>12.0 ± 1.0</td>
<td>11.7 ± 1.1</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure (mmHg)</td>
<td>18 ± 3</td>
<td>15 ± 3</td>
<td>19 ± 3</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.48 ± 0.01</td>
<td>7.50 ± 0.02</td>
<td>7.49 ± 0.01</td>
<td>7.55 ± 0.05</td>
</tr>
<tr>
<td>PO₂ (mmHg)</td>
<td>33 ± 2‡</td>
<td>31 ± 1</td>
<td>33 ± 2</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>98 ± 10‡</td>
<td>98 ± 7</td>
<td>99 ± 9</td>
<td>106 ± 9</td>
</tr>
</tbody>
</table>

Data are mean ± SEM of 7–10 animals. No difference over time in the clonidine + neostigmine group.

* Clonidine alone group differed significantly over time in mean arterial (P < 0.04) and right atrial pressure (P < 0.001).
† Between groups comparison: heart rate and right atrial pressure significantly different (P < 0.001).
‡ Significant variables similar except for cardiac output, PO₂, and PaO₂ (P < 0.02).
§ Significantly different from baseline.

Cardiovascular Effects

It may appear paradoxical that neostigmine, which decreases heart rate and blood pressure after intravenous administration, can increase these variables after intrathecal injection. Cardiovascular depression after intravenous administration results from amplification of the actions of vagally released acetylcholine, whereas cardiovascular stimulation after intrathecal administrations results from amplification of the actions of acetylcholine release on preganglionic sympathetic neurons, where it is an excitatory neurotransmitter. Mild cardiovascular stimulation has been observed after intrathecal neostigmine injection in sheep, although this effect was not statistically significant in the current study or in human volunteers. It would appear that the degree of cardiovascular stimulation from intrathecally administered neostigmine is greater in the rat than in sheep or humans, perhaps reflecting diminished penetration of neostigmine into the intermedio-lateral column in species with larger spinal cords.

Intrathecally administered α₁-adrenergic agonists cause cardiovascular depression by inhibiting preganglionic sympathetic nerve activity and by systemic redistribution to brainstem sites of sympathetic control. The 200-μg dose of clonidine chosen in the current study was previously shown to cause maximal hemodynamic depression in sheep. Larger doses decrease blood pressure less, because of direct peripheral vascular constriction from circulating drug, which may explain greater cardiovascular depression in this study than in a previous study of similar design in sheep employing a larger clonidine dose. Intrathecal neostigmine (1 mg) counteracts hypotension from intrathecally administered clonidine in conscious sheep, but this effect is maximal when neostigmine is administered 75 min before clonidine. In the current study, simultaneous administration of clonidine to a larger dose of neostigmine (2 mg) minimally altered clonidine-induced hemodynamic depression. It may be that sympathetic stimulation by intrathecally administered neostigmine is mild in species with a larger spinal cord, such as sheep, and that such counteraction may not be observed in humans after simultaneous administration of both drugs.
INTRATHECAL NEOSTIGMINE AND SPINAL CORD BLOOD FLOW

Fig. 1. Whole spinal cord blood flow (in ml·min⁻¹·10⁶ g⁻¹) in animals receiving 0.2 mg neostigmine (○), 2 mg neostigmine (●), 0.2 mg clonidine (▲), or 0.2 mg clonidine plus 2 mg neostigmine (▼) intrathecally after baseline measurements. Each symbol represents the mean ± SEM of four to seven animals. There were no differences in any group compared to baseline.

Respiratory and Behavioral Effects

Intrathecally administered neostigmine produced no evidence of significant respiratory depression, in agreement with previous studies in awake animals. Similarly, respiratory depression or hypoxemia have not been reported after intravenous administration of neostigmine.

Clonidine causes severe hypoxemia after bolus intravenous administration and can cause mild hypoxemia after bolus intraspinal administration in sheep. This is thought to be due to platelet aggregation caused by circulating clonidine, leading to pulmonary microembolism, and is peculiar to this species. Lack of reduction in arterial P_O₂ by clonidine in the current study likely reflects the small dose employed.

Intrathecal neostigmine could redistribute in cerebrospinal fluid to the brain, where it could cause a central cholinergic crisis, often manifested by hallucinations and agitation. These central nervous system symptoms can be observed after environmental exposure to cholinesterase inhibitors that cross the blood-brain barrier. Although behavioral signs of agitation were absent in the current study, initial phase I clinical safety assessments of intrathecal neostigmine should include monitoring for central cholinergic hyperactivity.

Intrathecal injection of muscarinic agonists or cholinesterase inhibitors can cause motor weakness after doses greater than those producing maximal antinociception. Although we did not test for subtle weakness in this study, a large dose (2 mg) of intrathecal neostigmine failed to elicit evidence of motor weakness. Similarly, motor blockade from intrathecal clonidine alone has not been observed in animals or humans.

Effects on Spinal Cord Blood Flow

Before discussion of these results, a few comments on methodologic issues are in order. First, this study used a relatively large number of colored microspheres over the course of the two separate experiments. There is a theoretical concern that the second day of microsphere injections might produce dissimilar results secondary to capillary occlusion from the first day's microsphere injections. This may be a particular concern for low-flow organs. However, the dosing regimen was randomized, and any possible effect of timing should be equalized. Second, this colored microsphere technique required more tissue preparation than the traditional method with radiolabeled microspheres. Compared to a study of similar design in this laboratory using radiolabeled microspheres, variability in measurement of the relatively low blood flows present in the spinal cord was greater with colored microspheres. However, there was not even a trend toward meaningful decreases in spinal cord blood flow after spinal neostigmine in this study. Third, this technique may not detect regional blood flow changes. However, as previously argued, the initial toxicologic question is whether a new drug causes generalized vasoconstriction, which would be reflected in global blood flow, as measured by this technique, rather than regional blood flow changes. Finally, this study did not include an intrathecal saline control experiment, because this has been shown not to affect cardiovascular variables or spinal cord blood flow in conscious sheep. Rather, the effect of neostigmine within the expected therapeutic dose range (0.2 mg) and ten times this dose were examined to increase the likelihood of observing any toxic effect.

Vascular endothelium in many vascular beds synthesizes the local vasodilator, nitric oxide, upon exposure to acetylcholine. It is possible that neostigmine could

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Table 3. Cervical Spinal Cord and Renal Cortical Blood Flows Following Intrathecal

<table>
<thead>
<tr>
<th>Group</th>
<th>Tissue</th>
<th>Baseline</th>
<th>+15 min</th>
<th>+60 min</th>
<th>+4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neostigmine, 0.2 mg</td>
<td>Right kidney</td>
<td>502 ± 86</td>
<td>450 ± 96</td>
<td>515 ± 72</td>
<td>559 ± 165</td>
</tr>
<tr>
<td></td>
<td>Left kidney</td>
<td>462 ± 50</td>
<td>486 ± 86</td>
<td>559 ± 74</td>
<td>593 ± 137</td>
</tr>
<tr>
<td></td>
<td>Cervical cord</td>
<td>7 ± 2</td>
<td>6 ± 1</td>
<td>8 ± 2</td>
<td>10 ± 4</td>
</tr>
<tr>
<td>Neostigmine, 2.0 mg</td>
<td>Right kidney</td>
<td>499 ± 121</td>
<td>579 ± 114</td>
<td>549 ± 88</td>
<td>527 ± 67</td>
</tr>
<tr>
<td></td>
<td>Left kidney</td>
<td>541 ± 122</td>
<td>582 ± 114</td>
<td>559 ± 82</td>
<td>555 ± 62</td>
</tr>
<tr>
<td></td>
<td>Cervical</td>
<td>7 ± 3</td>
<td>9 ± 3</td>
<td>8 ± 2</td>
<td>12 ± 5</td>
</tr>
<tr>
<td>Clonidine, 0.2 mg</td>
<td>Right kidney</td>
<td>706 ± 76</td>
<td>656 ± 87</td>
<td>612 ± 71</td>
<td>535 ± 76</td>
</tr>
<tr>
<td></td>
<td>Left kidney</td>
<td>467 ± 105</td>
<td>374 ± 88</td>
<td>382 ± 71</td>
<td>314 ± 73</td>
</tr>
<tr>
<td></td>
<td>Cervical cord</td>
<td>12 ± 3</td>
<td>12 ± 4</td>
<td>10 ± 4</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Clonidine 0.2 mg +</td>
<td>Right kidney</td>
<td>616 ± 92</td>
<td>697 ± 89</td>
<td>590 ± 76</td>
<td>675 ± 110</td>
</tr>
<tr>
<td>neostigmine, 2.0 mg</td>
<td>Left kidney</td>
<td>384 ± 90</td>
<td>350 ± 116</td>
<td>325 ± 69</td>
<td>385 ± 108</td>
</tr>
<tr>
<td></td>
<td>Cervical cord</td>
<td>15 ± 5</td>
<td>14 ± 4</td>
<td>10 ± 7</td>
<td>12 ± 3</td>
</tr>
</tbody>
</table>

Data are mean ± SEM of 5–7 animals. Blood flows expressed in ml·min⁻¹·100 g⁻¹. No significant differences among groups. No significant differences within groups over time.

have increased spinal cord blood flow by increasing acetylcholine concentrations and hence acetylcholine-induced nitric oxide synthesis. Nearly 20% of nitric oxide synthase in sheep spinal cord resides in the particulate fraction after high-speed centrifugation and likely represents endothelial nitric oxide synthase. However, with the doses of neostigmine used in the current study, no increase in spinal cord blood flow was observed.

This study confirms previous observations that intraspinal clonidine does not reduce spinal cord blood flow. As important is that spinal cord blood flow was not affected by the combination of neostigmine and clonidine, which might prove clinically useful for enhancement of clonidine analgesia.

In summary, intrathecal neostigmine did not affect cardiorespiratory variables or spinal cord blood flow. Clonidine alone decreased heart rate and systemic arterial and central venous pressure, whereas the addition of neostigmine to clonidine only partially counteracted this effect. Clonidine alone or with neostigmine did not affect spinal cord blood flow. These observations, coupled with detailed behavioral and histopathologic studies in rats and dogs, support phase 1 safety assessment of intrathecal neostigmine in humans.

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References


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