

## Intrathecal Amitriptyline

### Antinociceptive Interactions with Intravenous Morphine and Intrathecal Clonidine, Neostigmine, and Carbamylcholine in Rats

James C. Eisenach, M.D.,\* G. F. Gebhart, Ph.D.†

**Background:** Systemically administered opioids induce analgesia in part by spinal noradrenergic, serotonergic, and cholinergic mechanisms. The current study tested whether antinociception from systemically administered opioids could therefore be enhanced by intrathecal injection of a monoamine reuptake inhibitor to potentiate the action of spinally released norepinephrine and serotonin (amitriptyline) and intrathecal injection of a cholinesterase inhibitor to potentiate the action of spinally released acetylcholine (neostigmine).

**Methods:** Rats were prepared with chronic lumbar intrathecal and femoral intravenous catheters and nociceptive threshold was assessed by hind paw withdrawal to a radiant heat stimulus. An isobolographic design was used to distinguish between additive and synergistic interactions.

**Results:** Intravenous morphine and intrathecal neostigmine, but not intrathecal amitriptyline, caused dose-dependent antinociception alone. Combining any two of these three treatments yielded a synergistic interaction compared to each alone, whereas combining all three yielded an additive interaction compared to each two-way interaction. Intrathecal amitriptyline did not affect antinociception from intrathecal clonidine or intrathecal carbamylcholine.

**Conclusions:** These data suggest that intrathecal doses of amitriptyline resulting in potentiation of intravenous morphine antinociception may not be adequate to block muscarinic receptors, because they did not affect carbamylcholine-induced antinociception. These results further support the relevance of spinal monoamine reuptake and cholinesterase inhibition to synergistically enhance analgesia from systemic opioids. (Key words: Analgesics, opioid: morphine. Antagonists, acetylcholinesterase: neostigmine. Antidepressants:

amitriptyline. Drug interaction: synergy. Drug interaction, analysis: isobologram. Receptors, spinal cord:  $\alpha_2$ -adrenergic; muscarinic; nicotinic.)

ALTHOUGH opioids can induce analgesia by actions in the periphery, the brain, and the spinal cord, attention has focused on the relevance of bulbospinal pathways in the action of clinically used systemic doses of opioids. Building on classic studies by Shiomi and Takagi<sup>1</sup> and Dahlstrom and Fuxe,<sup>2</sup> several anatomic, neurochemical, and neurophysiologic lines of evidence support activation of bulbospinal serotonergic and noradrenergic pathways as being relevant to analgesia induced by systemically administered opioids.<sup>3-9</sup> More recently, spinal cholinergic mechanisms of analgesia from systemic opioids have been described,<sup>10-12</sup> and some studies suggest a link between opioid-induced bulbospinal noradrenergic stimulation and spinal cholinergic interneuron activation.<sup>13,14</sup>

Although previous studies have shown that intrathecal administration of monoamine reuptake inhibitors can enhance antinociception from systemic opioids,<sup>15,16</sup> dose-response characteristics and isobolographic interaction studies have not been performed. In addition, whether intrathecal administration of cholinesterase inhibitors enhances systemic opioid analgesia, and whether opioid analgesia can be further amplified by simultaneous inhibition of monoamine reuptake and cholinesterase have not been examined. The purpose of the current study was to examine, using isobolographic techniques, the interactions among intravenous morphine, intrathecal neostigmine, and intrathecal amitriptyline in rats. In addition, because amitriptyline exhibits muscarinic antagonist properties<sup>17,18</sup> that could diminish enhancement of intravenous morphine, we tested whether intrathecal amitriptyline in doses that enhanced intravenous morphine affected antinociception from intrathecal carbamylcholine. Finally, because amitriptyline could enhance the analgesic ac-

\* Professor, Department of Anesthesia, Wake Forest University Medical Center, Winston-Salem, North Carolina.

† Professor, Department of Pharmacology, University of Iowa, Iowa City, Iowa.

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



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tion of spinally administered  $\alpha_2$ -adrenergic agonists by inhibiting serotonin reuptake, we tested whether intrathecal amitriptyline increased antinociception from intrathecal clonidine.

## Materials and Methods

### Animals

After obtaining approval by the Animal Care and Use Committee, 48 male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 325–375 g were studied. Rats were surgically prepared under sodium pentobarbital anesthesia (40–50 mg/kg, intraperitoneally). A femoral venous catheter was implanted for morphine administration, exteriorized, and secured at the back of the head. An intrathecal catheter (PE-10 tubing) was inserted through a small opening in the cisterna magnum and passed 8.5 cm caudad in the intrathecal space. After surgery, rats were housed individually with free access to food and water and allowed to recover for at least 1 week before use. Rats showing postoperative neurologic deficits were killed immediately. Each animal was studied two or three times in an experimental series, with a 2–5-day interval between studies. Separate analysis of response to treatments *versus* time from catheter insertion demonstrated no effect of time of testing.

### Nociceptive Testing

The hind paw thermal withdrawal was used as previously described<sup>19</sup> to test thermal antinociception. Briefly, the intrathecal and intravenous catheters were connected to PE-20 tubing and syringes prefilled with all drugs to be administered during the study, and the rats were placed in a clear plastic container on a raised floor of clear, heat-tempered glass. After 15–30 min for the animal to become habituated to the environment, a radiant heat source (50 W halogen projector lamp, GTE Products Corp, Winchester, KY), with bulb intensity controlled by a constant voltage source, was focused on the plantar surface of one hind paw where it was in contact with the glass (distance from end of focus cone to paw was 0.7 cm). Bulb intensity was adjusted so that the baseline latency to paw withdrawal from the heat source was 10–15 s. Both paws were tested in random order 1–2 min apart, and the average of their values was calculated. Cutoff time in the absence of a response was 30 s to avoid tissue damage.

### Drugs and Their Administration

Carbamylcholine, mecamlamine, and morphine sulfate were obtained from Sigma Chemical Co. (St Louis, MO). Neostigmine methylsulfate was obtained from International Medication Systems, Ltd (El Monte, CA). Amitriptyline was obtained from Stuart Pharmaceutical Co. (Wilmington, DE). Clonidine hydrochloride was donated by Boehringer-Ingelheim (Ridgefield, CT). All drugs were dissolved in normal saline, with pH levels between 6.4 and 7.8. All drugs except morphine were injected intrathecally over 30 s in a volume of 3  $\mu$ l followed by a 10- $\mu$ l flush. Morphine was injected intravenously in a volume of 0.3 ml followed by a 0.3-ml saline flush. In preliminary experiments, we confirmed that all drugs reached peak effects within 5–10 min and their effects were sustained for 30–45 min.

### Experimental Paradigm

On study days, catheters were connected, the rat was habituated to the testing environment, and baseline hind paw withdrawal latencies were obtained. In single agonist studies, animals then received cumulative dosing, at 15-min intervals, of intravenous morphine (3, 7, 12 mg/kg cumulative dose), intrathecal amitriptyline (20, 60  $\mu$ g), neostigmine (1, 3, 7, 12  $\mu$ g), carbamylcholine (1, 3, 10  $\mu$ g), or clonidine (0.5, 1, 3, 10  $\mu$ g), with hind paw withdrawal latencies determined every 5 min, and values obtained at 10 and 15 min after each dose averaged for the value for that dose. In the isobolographic studies, fixed ratio combinations were administered in a cumulative dose response with the following ratios: for intrathecal amitriptyline plus intravenous morphine 5  $\mu$ g:1000  $\mu$ g/kg; 10  $\mu$ g:1000  $\mu$ g/kg; and 20  $\mu$ g:1000  $\mu$ g/kg; for intrathecal neostigmine plus intravenous morphine 1  $\mu$ g:1000  $\mu$ g/kg; for intrathecal amitriptyline plus intrathecal neostigmine 20  $\mu$ g:1  $\mu$ g; and for intrathecal amitriptyline plus intrathecal clonidine 40  $\mu$ g:1  $\mu$ g. These ratios were chosen to approximate relative potencies determined from single agonist studies above. In the case of one agent lacking efficacy alone, the ratio chosen in an isobolographic study is somewhat arbitrary. In these studies, the upper limit of amitriptyline used, 60  $\mu$ g total cumulative dose, was the highest dose that, in pilot experiments, did not result in transient hind limb motor dysfunction. Two lower doses, one half and one quarter this dose, were also evaluated in combination with morphine. To examine the three-way interaction, intrathecal amitriptyline, intrathecal neostigmine, and



intravenous morphine were administered in a fixed ratio of 14  $\mu\text{g}$ :0.26  $\mu\text{g}$ :1000  $\mu\text{g}/\text{kg}$ . This ratio was chosen to approximate the relative potencies of one combination (intrathecal amitriptyline plus intrathecal neostigmine) with another (intrathecal amitriptyline plus intravenous morphine).

Two antagonist studies were performed. To test whether biting/scratching activity after injection of the amitriptyline/neostigmine combination was due to nicotinic receptor stimulation, intrathecal mecamlamine, 10  $\mu\text{g}$ , was injected 10 min before intrathecal neostigmine, 0.5  $\mu\text{g}$  plus intrathecal amitriptyline, 10  $\mu\text{g}$ . To test whether amitriptyline functioned as a muscarinic receptor antagonist, 60  $\mu\text{g}$  intrathecal amitriptyline, was injected 15 min before cumulative dosing with carbamylcholine.

#### Statistics

Data are presented as mean  $\pm$  SEM. Because a cutoff value was used, data were converted to percent maximum possible effect according to the formula:

percent maximum possible effect = (observed - baseline)/(30 s - baseline)  $\times$  100

Effective dose 50 ( $\text{ED}_{50}$ ) was defined as the dose that yielded a 50% maximum effect.  $\text{ED}_{50}$  and 95% confidence intervals were calculated by a graded  $\text{ED}_{50}$  program (developed at the University of Iowa<sup>20</sup>).

Isobolographic analysis at the  $\text{ED}_{50}$  level for two-way drug interactions was conducted according to the procedure of Tallarida *et al.*,<sup>21</sup> and, in the case of interactions involving intrathecal amitriptyline, according to a modified method described by Porreca *et al.*<sup>22</sup> in which one drug lacks efficacy. Confidence intervals for each point were calculated from the variances of each component alone. Confidence intervals were evaluated for statistical significance with a Student's *t* test. A value of  $P < 0.05$  was considered significant. Isobolographs were constructed for each two-way interaction, and, for the three-way interaction, a three-dimensional isobolograph was constructed. In the case of a three-way interaction, there is a plane of additivity, rather than a line of additivity. Two such planes of additivity are possible: one described by the  $\text{ED}_{50}$ 's of each single component, and another described by the  $\text{ED}_{50}$ 's of each fixed ratio combination of two components. In each case, a theoretically additive point is calculated from the ratio chosen and the relative potencies of each drug, and its variance determined as an extension of the method for a two-component point.<sup>21</sup>

To quantify the strength of these interactions, an algebraic (fractional) method of drug interaction at the  $\text{ED}_{50}$  level was used. As applied to this type of antinociceptive paradigm by Naguib and Yaksh,<sup>23</sup> this involves the expression of the component doses of the two agents (or 3 in one case) given jointly as fractions of the doses that produce the same effect when given separately. The sum of the fractional doses is determined as

$$da/Da + db/Db + dc/Dc,$$

where  $D_a$ ,  $D_b$ , and  $D_c$  are the  $\text{ED}_{50}$  values of agents  $a$ ,  $b$ , and  $c$  given alone, and  $da$ ,  $db$ , and  $dc$  are the doses of  $a$ ,  $b$ , and  $c$  that, when combined, are equipotent with  $D_a$ ,  $D_b$ , or  $D_c$ . Values less than 1 imply a synergistic interaction, and the lower the value, the more powerful the interaction.

## Results

### Single Agonist Studies

All treatments except intrathecal amitriptyline resulted in a dose-dependent increase in the thermal withdrawal latency (fig. 1). The rank order of potency (and  $\text{ED}_{50}$ ) was clonidine (1.6  $\mu\text{g}$ ) = carbamylcholine (1.7  $\mu\text{g}$ ) > neostigmine (3.7  $\mu\text{g}$ ); > intravenous morphine (6700  $\mu\text{g}/\text{kg}$ ); and > amitriptyline (no effect). Neostigmine and carbamylcholine were associated with tail grooming and licking behavior, clonidine was associated with urination, and intravenous morphine was

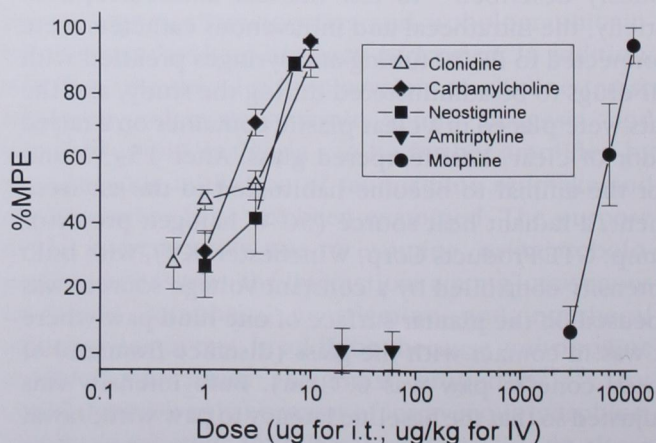


Fig. 1. Percent maximal possible antinociceptive effect after intrathecal injection of clonidine ( $\Delta$ ), carbamylcholine ( $\blacklozenge$ ), neostigmine ( $\blacksquare$ ), amitriptyline ( $\blacktriangledown$ ), or intravenous morphine ( $\bullet$ ). Each value represents the mean  $\pm$  SEM of six animals.



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associated, at the highest dose, with cessation of exploring activity.

### Double Combination Studies

In the special case of an agent lacking efficacy, any statistically significant decrease in the  $ED_{50}$  of the other, active component denotes synergy. As such, intrathecal amitriptyline synergistically enhanced antinociception from intravenous morphine (fig. 2). For the ratios examined, there was no difference in reduction in the  $ED_{50}$  of intravenous morphine with fixed amitriptyline: morphine ratios of 5, 10, and 20  $\mu$ g amitriptyline: 1,000  $\mu$ g/kg morphine (fig. 2 and table 1). Behavioral inactivity observed with large intravenous doses of morphine alone was not observed in these combination experiments, although the largest morphine dose administered was 7 mg/kg.

Intrathecal amitriptyline also synergistically enhanced antinociception from intrathecal neostigmine (fig. 3 and table 1). In addition to the grooming behavior associated with intrathecal neostigmine, animals exhibited scratching and biting of the flank at the low and intermediate dose of the amitriptyline:neostigmine combination. Rats did not, however, vocalize or exhibit truncal rigidity, nor did they exhibit unusual behavior when lightly stroked or in response to noise. In separate studies, intrathecal pretreatment with the nicotinic receptor antagonist, mecamylamine, had no effect on this behavior, nor did it affect antinociception, which was  $65 \pm 8\%$  maximum possible effect from intrathecal neostigmine, 0.5  $\mu$ g, plus intrathecal amitriptyline, 10  $\mu$ g alone, and  $56 \pm 7\%$  maximum possible effect from this combination 10 min after mecamylamine administration.

Intrathecal amitriptyline did not affect antinociception from intrathecal clonidine (fig. 4 and table 1), in contrast to its effect on morphine and neostigmine. Amitriptyline did not alter urination after exposure to intrathecal clonidine. A fixed dose (60  $\mu$ g) of amitriptyline did not alter dose-dependent antinociception from intrathecal carbamylcholine (fig. 5), nor did it alter grooming behavior after administration of intrathecal carbamylcholine.

Intrathecal neostigmine synergistically enhanced antinociception from intravenous morphine (fig. 6 and table 1). Behavioral inactivity observed with large intravenous doses of morphine alone was not observed in this combination, although the largest morphine dose administered was 6 mg/kg. Morphine had no apparent effect on the neostigmine-induced tail-grooming behavior.

Table 1.  $ED_{50}$  Values and 95% Confidence Intervals for Drug Combinations

Group	Amitriptyline Component		Morphine Component		Neostigmine Component		Clonidine Component		Sum of $ED_{50}$ Fractions
	Fraction of $ED_{50}$	Intrathecal Dose ( $\mu$ g)	Fraction of $ED_{50}$	Intravenous Dose ( $\mu$ g/kg)	Fraction of $ED_{50}$	Intrathecal Dose ( $\mu$ g)	Fraction of $ED_{50}$	Intrathecal Dose ( $\mu$ g)	
NEO + MOR (1:1,000)	—	—	0.12 (0.04–0.20)	810 (260–1,400)	0.22 (0.07–0.37)	0.81 (0.26–1.4)	—	—	0.34 (0.11–0.57)
AMI + CLO (40:1)	0	52 (28–76)	—	—	—	—	0.79 (0.41–1.1)	1.3 (0.69–1.9)	0.79 (0.41–1.1)
AMI + MOR (5:1,000)	0	10 (6.1–14)	0.30 (0.18–0.42)	2,000 (1,200–2,800)	—	—	—	—	0.30 (0.18–0.42)
AMI + MOR (10:1,000)	0	14 (13–16)	0.24 (0.21–0.27)	1,600 (1,400–1,800)	—	—	—	—	0.24 (0.21–0.27)
AMI + MOR (20:1,000)	0	45 (36–54)	0.34 (0.27–0.41)	2,300 (1,800–2,700)	—	—	—	—	0.34 (0.27–0.41)
AMI + NEO (20:1)	0	6.6 (3.8–9.4)	—	—	0.09 (0.05–0.13)	0.33 (0.19–0.47)	—	—	0.09 (0.05–0.13)
AMI + MOR + NEO	0	13 (8.7–16)	0.13 (0.09–0.17)	870 (610–1,100)	0.06 (0.04–0.08)	0.23 (0.15–0.31)	—	—	0.19 (0.13–0.25)

Values are means of 5–7 animals; 95% confidence intervals are in parentheses. AMI = amitriptyline; CLO = clonidine; MOR = morphine; NEO = neostigmine.



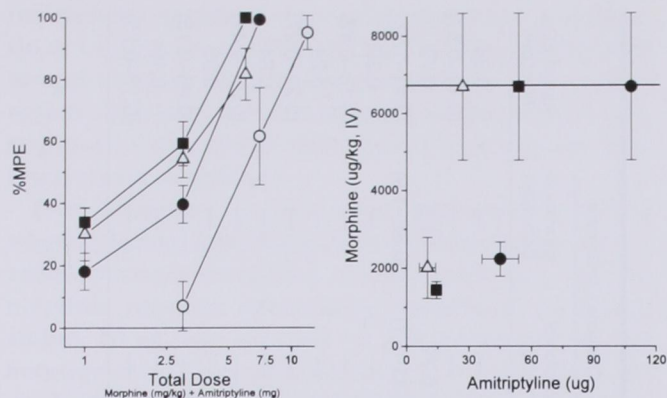


Fig. 2. Percent maximal possible antinociceptive effect after intravenous injection of morphine alone (○) or in fixed ratio combination with intrathecal injection of amitriptyline 5:1000 (△), 10:1000 (■), or 20:1000 (●). Each value represents the mean  $\pm$  SEM of six animals. The total dose injected (morphine alone or morphine + amitriptyline) for each data point is shown on the x-axis of the left panel. The right panel represents the isobologram at the ED<sub>50</sub> level for interaction between intravenous morphine and intrathecal amitriptyline. In the case of an agent lacking efficacy alone (amitriptyline, the line of additivity is a horizontal line, and the theoretical additive points and confidence intervals for each ratio lie on that line.

#### Triple Combination Study

Simultaneous administration of intravenous morphine, intrathecal amitriptyline, and intrathecal neostigmine resulted in a synergistic interaction compared to each agent alone (fig. 7, right). However, the ED<sub>50</sub> of the triple combination lay directly on the theoretical plane of additivity defined by each two-way combina-

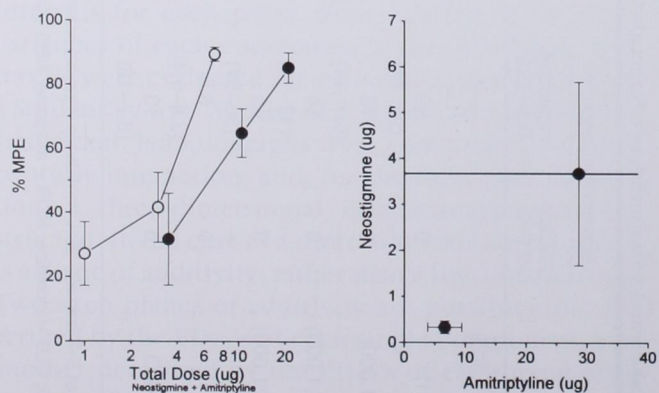


Fig. 3. Percent maximal possible antinociceptive effect after intrathecal injection of neostigmine alone (●) or in fixed ratio combination with intrathecal injection of amitriptyline (○). Each value represents the mean  $\pm$  SEM of six animals. The right panel represents the isobologram for interaction between intrathecal neostigmine and amitriptyline.

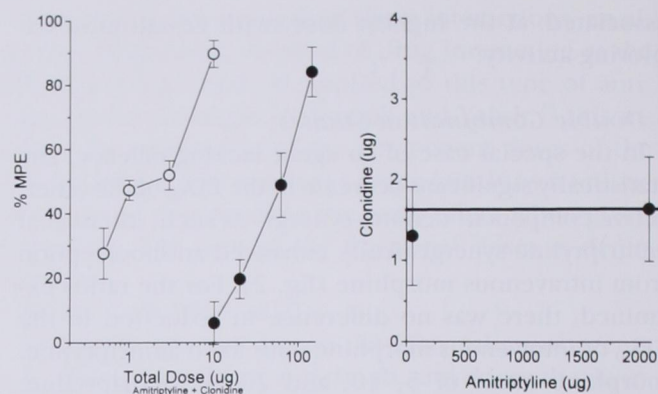


Fig. 4. Percent maximal possible antinociceptive effect after intrathecal injection of clonidine alone (○), or in a fixed ratio combination with intrathecal clonidine and intrathecal amitriptyline (●). Each value represents the mean  $\pm$  SEM of six animals. Panel on the right represents the isobologram for interaction between intrathecal clonidine and intrathecal amitriptyline.

tion (fig. 7, left), indicating no supra-additive enhancement of any two agents by the third. The doses of neostigmine and morphine were, however, lower to achieve an ED<sub>50</sub> with amitriptyline than without as a consequence of this additive interaction (table 1). Animals receiving this triple combination exhibited no unusual behaviors peculiar to each component alone, although maximal doses tested were low for each individual component.

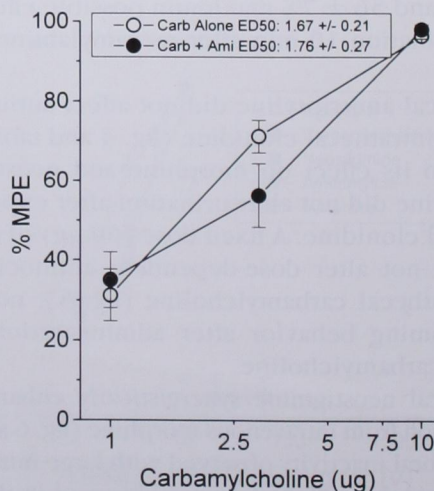


Fig. 5. Percent maximal possible antinociceptive effect after intrathecal injection of carbamylcholine alone (○), or after pretreatment with intrathecal amitriptyline, 60 µg (●). Each value represents the mean  $\pm$  SEM of six animals.



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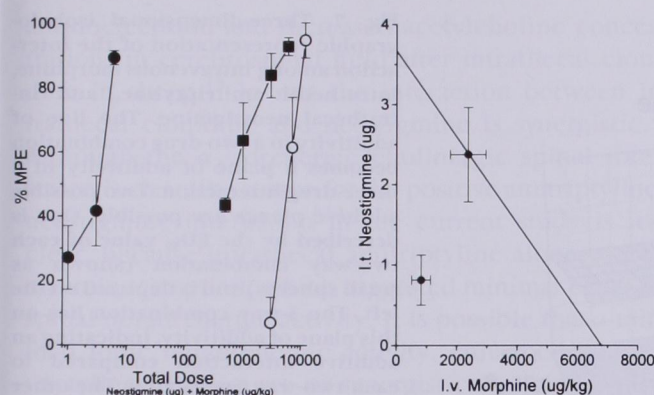


Fig. 6. Percent maximal possible antinociceptive effect after intravenous injection of morphine alone (○), intrathecal injection of neostigmine alone (●), or a fixed ratio combination with intravenous morphine and intrathecal injection of neostigmine (■). Each value represents the mean  $\pm$  SEM of six animals. The right panel represents the isobologram for interaction between intravenous morphine and intrathecal amitriptyline.

## Discussion

These data provide the first quantitative evidence of interaction between intrathecal amitriptyline and intravenous morphine and the first evidence of any kind of interaction between intrathecal neostigmine and intravenous morphine. In addition, these results support the following hypotheses of mechanisms of interaction among these agents and their clinical application.

### Opioid-induced Descending Inhibition and Spinally Administered Drugs

A variety of lines of evidence support activation of bulbospinal noradrenergic and serotonergic inhibitory pathways in the analgesic action of opioids. For example, systemically administered opioids increase norepinephrine in lumbar cerebrospinal fluid in animals<sup>14</sup> and humans,<sup>‡</sup> an effect that is blocked by naloxone. Similarly, systemically administered opioids increase norepinephrine in microdialysis samples of the dorsal, but not ventral horn, an effect that is blocked by cervical spinal cord transection,<sup>24</sup> and systemically administered opioids increase serotonin metabolites in human cerebrospinal fluid.<sup>‡</sup> Responses of dorsal horn neurons to peripheral nociceptive stimuli are reduced

by opioid injection or stimulation in brain stem areas known to contain serotonergic neurons with descending projections and with projections to noradrenergic neurons with descending projections, and this inhibition is reversed by noradrenergic and serotonergic receptor antagonists.<sup>7,9,25</sup> Finally, antinociception from systemically administered opioids is inhibited by spinally administered noradrenergic and serotonergic receptor antagonists.<sup>3,26-29</sup>

Although previous studies have demonstrated an enhancement of antinociception from systemic opioids by acutely administered intrathecal injection of amitriptyline,<sup>16</sup> this is the first examination of this interaction using isobolographic analysis. The results demonstrate a threefold increase in potency of intravenous morphine over a wide range of intrathecal amitriptyline doses, corroborating previous single-dose studies and the cited pharmacologic literature.

Ethical reasons have hampered the clinical study of interactions between opioids and amitriptyline or other tricyclic antidepressants because of the inability to inject these monoamine reuptake inhibitors intrathecally. Thus, systemically administered inhibitors of the reuptake of norepinephrine, serotonin, or both have been shown to have minor effects on systemic opioid analgesia in humans, with results indicating potentiation, no effect, or antagonism, depending on the agent, timing of drug administration, and type of pain.<sup>30-34</sup> This is not surprising, given the complex action of these agents at multiple sites when administered systemically, especially over prolonged periods. In contrast, the current results suggest a clear potentiation of systemic opioid antinociception when a mixed inhibitor of monoamine reuptake is administered intrathecally.

Spinal cholinergic mechanisms have also been implicated in analgesia from systemically administered opioids. For example, intravenous morphine increases acetylcholine concentrations in lumbar cerebrospinal fluid of animals<sup>14</sup> and humans (JCE: unpublished observations) and antinociception from intravenous morphine is inhibited by intrathecal injection of atropine.<sup>12</sup> Anatomic studies suggest cholinergic stimulation is caused by activation, by descending systems, of spinal cholinergic interneurons, whose cell bodies are located in the neck of the dorsal horn and surrounding the central canal, and whose fibers ascend to the superficial dorsal horn.<sup>35</sup> Antinociception from intrathecal injection of cholinergic agonists is a result of muscarinic stimulation, because it is inhibited by muscarinic, but

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



## Compared to:

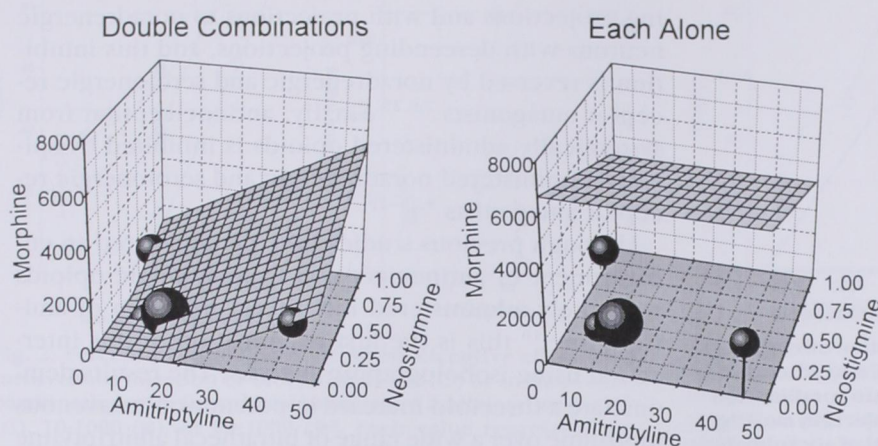


Fig. 7. Three-dimensional isobolographic representation of the interaction among intravenous morphine, intrathecal amitriptyline, and intrathecal neostigmine. The line of additivity in a two-drug combination becomes a plane of additivity in a three-drug interaction. Two possible additive planes are possible. One is described by the  $ED_{50}$  value of each two-way combination (shown as small spheres) and is depicted on the left. The 3-way combination lies on this plane of additivity, indicating an additive interaction compared to each two-way interaction. The other possible additive plane is that described by the  $ED_{50}$  value of each agent alone, shown at right. Each two-way interaction, again depicted as a small sphere, lies significantly below this sphere, indicating synergy. Similarly, the three-way combination, shown as the large sphere, lies significantly below the theoretical plane of additivity, demonstrating synergy compared to each agent alone.

not nicotinic receptor antagonists.<sup>36</sup> The majority of muscarinic receptors in the superficial dorsal horn are demonstrated to be presynaptic on fine afferent fibers.<sup>37</sup> It therefore has been speculated that activation of these receptors diminishes stimulus-evoked excitatory neurotransmitters from nociceptive afferents.<sup>38</sup>

Intrathecal administration of neostigmine alone caused antinociception in the current study, similar to previous observations in rats<sup>23</sup> and humans.<sup>39</sup> In these species, therefore, there is likely to be tonic spinal cholinergic activity, as has been demonstrated in rats.<sup>40</sup> This is the first study to test the hypothesis that intravenous opioid antinociception would be enhanced by intrathecal injection of a cholinesterase inhibitor, although this follows logically from the antagonist studies outlined earlier. This study also demonstrates a synergistic interaction between intravenous morphine and intrathecal neostigmine, which would be expected if morphine is acting in part *via* spinal acetylcholine release and if inhibition of acetylcholine breakdown would produce a non-linear enhancement of the postsynaptic effects of morphine-induced acetylcholine release. These results provide the rationale for examining this interaction clinically, which is now possible because intrathecal neostigmine has been introduced into clinical trials.

#### Minimal Antinociception with Intrathecal Amitriptyline without Muscarinic Blockade

The current study agrees with previous work<sup>41</sup> demonstrating no or minor analgesia from acutely administered systemic or intrathecal amitriptyline. As such, these results do not support a tonically active release of norepinephrine or serotonin in the spinal cord of the awake, uninjured rat. In addition, amitriptyline did not enhance antinociception from intrathecal clonidine in the current study. This observation further supports lack of tonic spinal release of norepinephrine or serotonin, because one would have expected an additive enhancement in the former case, because both norepinephrine and clonidine act on  $\alpha_2$ -adrenoceptors, and a synergistic enhancement in the latter case, because there is a synergistic interaction between serotonergic and  $\alpha_2$ -adrenergic agonists.<sup>42</sup>

Intrathecal administration of amitriptyline did, however, synergistically enhance intrathecal neostigmine-induced antinociception. It is possible that this synergism is due to increased  $\alpha_2$ -adrenergic stimulation from amitriptyline and cholinergic stimulation from neostigmine. There may be a link between spinally released norepinephrine, acting on  $\alpha_2$ -adrenergic receptors, and acetylcholine. For example, antinociception from intrathecal clonidine is diminished by intrathecal atropine in rats,<sup>43</sup> and intrathecal neostigmine enhances



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antinociception and increased acetylcholine concentrations in cerebrospinal fluid after intrathecal clonidine in sheep.<sup>13</sup> In rats the interaction between intrathecal clonidine and neostigmine is synergistic.<sup>23</sup> Although the  $\alpha_2$ -adrenergic/cholinergic spinal interaction is clear, the reason for the positive amitriptyline/neostigmine interaction in the current study is less clear, because intrathecal amitriptyline alone caused no antinociception, which suggested minimal effect on spinal noradrenergic activity. It is possible that a minimal tonic noradrenergic activity, enhanced by amitriptyline, is adequate to synergistically increase the effect of neostigmine, or that some other property of amitriptyline is responsible for this enhancement.

Amitriptyline is a potent muscarinic receptor antagonist, competing for muscarinic ligand binding in low micromolar concentrations,<sup>17</sup> and this property is responsible for the considerable side effects observed with this agent during systemic use. Two observations from the current study suggest amitriptyline, administered intrathecally in doses that enhance other analgesics, does not produce significant muscarinic receptor blockade. First is the synergistic interaction with neostigmine, which, as discussed earlier, is of an undetermined mechanism, but clearly could not involve muscarinic receptor antagonism, because such antagonists inhibit rather than enhance neostigmine-induced analgesia.<sup>23</sup> Second is the direct observation that the maximal dose of intrathecal amitriptyline employed in the current series of studies had no effect on intrathecal carbamylcholine-induced antinociception, which also has been demonstrated to be inhibited by intrathecal muscarinic receptor antagonists.<sup>36</sup>

### Clinical Implications

It is inappropriate to administer amitriptyline intrathecally to humans: no toxicologic assessment has been performed, the drug is available commercially only in a form containing preservatives, and motor weakness observed with intrathecal amitriptyline doses of  $>60 \mu\text{g}$  could have represented early neurotoxicity, although all rats recovered full motor function as determined by gross observation. Nonetheless, the current study provides important reasons to examine the pre-clinical toxicity of amitriptyline for spinal use in humans.

As discussed earlier, drug-induced side effects remain a barrier to easy and effective pain therapy, and one approach to reduce such side effects is to combine small doses of analgesics of other types. Of particular interest

are drugs that interact synergistically, because a larger reduction in the dose of each component is possible. These positive interactions are useful; however, negative interactions would be caused if the drugs enhance each other's side effects.

Intravenous morphine and intrathecal amitriptyline and neostigmine each produced unique side effects in the current study. Although side effects were only studied by gross behavioral assessment, lack of morphine-induced inactivity when morphine was combined with these other agents suggests that these combinations do not enhance this effect of morphine. Tail grooming behavior after intrathecally administered muscarinic receptor agonists and cholinesterase inhibitors in rats have been described previously. The more exaggerated scratching behavior observed when amitriptyline was combined with neostigmine is suggestive of a pronociceptive effect observed with a neostigmine/atropine combination in rats, due to unopposed nicotinic receptor stimulation.<sup>23</sup> However, in contrast to that syndrome, allodynia was not present, there was no spontaneous vocalization, and the behavior was unaffected by intrathecal injection of the nicotinic receptor antagonist, mecamylamine. Nonetheless, there remains the possibility that the neostigmine/amitriptyline combination may cause dysesthesias in humans.

Finally, this study provides the rationale for inhibition of reuptake or metabolism of spinal norepinephrine, serotonin, and acetylcholine as an efficient method to enhance analgesia from intravenous morphine. Thus, inhibition of monoamine reuptake by amitriptyline and cholinesterase by neostigmine increased the potency of intravenous morphine sixfold. No behavioral side effects were present on simple observation in animals receiving the lower doses of these three compounds in combination, suggesting such an approach could enhance opioid analgesia clinically while reducing side effects.

In summary, intrathecal administration of amitriptyline alone causes no antinociception to a noxious heat stimulus in awake rats, but synergistically enhances antinociception from intravenous morphine and intrathecal neostigmine. Lack of inhibition of antinociception from intrathecal carbamylcholine by amitriptyline suggests that, at doses that enhance other analgesics, intrathecal amitriptyline does not antagonize muscarinic actions. These results support the hypothesis that morphine antinociception may be potentiated by simultaneous inhibition of removal of cascading inhibitory spinal neurotransmitters released by morphine.



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