

Sex Differences in Cholinergic Analgesia II

Differing Mechanisms in Two Models of Allodynia

Patricia M. Lavand'homme, M.D.,* James C. Eisenach, M.D.†

Background: Cholinergic agents reduce allodynia after nerve injury in animals and may be useful in the treatment of neuropathic pain. Intrathecally administered neostigmine and neuronal nicotinic agonists are more potent in female than in male rats against acute thermal noxious stimuli. The purpose of this study was to determine whether there is also a sex difference in the antiallodynic effects of intrathecal cholinomimetic agents in two models of allodynia and to test their pharmacologic mechanisms.

Methods: Male and female rats with indwelling intrathecal catheters received injections of neostigmine, bethanechol (muscarinic agonist), RJR-2403 (neuronal nicotinic agonist) alone or with atropine (muscarinic antagonist), mecamylamine (nicotinic antagonist), phentolamine (α -adrenergic antagonist), or saline control. The effect of these agents was determined on mechanical allodynia produced by either intraplantar injection of capsaicin or ligation of spinal nerves.

Results: Neostigmine and RJR-2403 but not bethanechol were more potent in female than in male rats in reducing allodynia after nerve injury, and antagonist studies were also consistent with a nicotinic component to explain this sex difference. Phentolamine did not reverse neostigmine's effect. In contrast, for capsaicin-induced allodynia, neostigmine plus mecamylamine but not neostigmine or RJR-2403 was more potent in female than in male rats.

Conclusions: These data demonstrate a sex difference of intrathecal neostigmine after nerve injury-induced allodynia similar to that observed in normal animals that received acute noxious thermal stimulation. However, this sex difference is not universal to all pain models because it was not present after

intradermal capsaicin injection, nor is its interaction with spinal noradrenergic mechanisms consistent in all models. (Key words: Chronic pain; intrathecal; muscarinic; neostigmine; neuropathic pain; noradrenergic; women's health.)

SOME chronic painful conditions are more prevalent among women than men,¹ particularly complex regional pain syndrome and chronic low back pain. Similarly, female rats are more likely than males to develop signs of neuropathic pain in certain models of neuropathic pain.² In humans, sex differences in pain prevalence emerge at adolescence when hormonal status changes,³ and in animals, some studies suggest that hormonal status greatly influences behavioral and pharmacologic responses to stress and pain.⁴ We speculate that part of this hormonal dependence may, in part, reflect sex differences in spinal cholinergic activity and have demonstrated antinociception from intrathecal neostigmine, which is more potent in normal female than male rats exposed to acute noxious heat stimuli.⁵ That study suggested that, in the normal animal, there is an increased sensitivity to spinal nicotinic receptor activation in females compared with males, which explains the sex difference in sensitivity to neostigmine. Because the effect of a spinal nicotinic agonist was partially reversed by phentolamine, and because nicotinic agonists are known to stimulate norepinephrine release,⁶ we proposed that a nicotinic-noradrenergic interaction was present in females and not in males.

Chronic pain differs in physiology and pharmacology to responses to acute noxious somatic stimulation, and it is not clear whether results in normal animals would apply to those with hypersensitivity. The purpose of this study was to test whether a sex difference in sensitivity to intrathecal neostigmine exists in animals with mechanical allodynia after spinal nerve ligation⁷ and after intraplantar injection of capsaicin.⁸ We chose both models because they are well characterized anatomically and pharmacologically and are claimed to be predictive of response in patients with neuropathic pain. In addition,

* Research Fellow, Department of Anesthesiology, Universite Catholique de Louvain, Brussels, Belgium.

† F. M. James III Professor, Department of Anesthesiology, Wake Forest University School of Medicine, Winston-Salem, North Carolina.

Received from the Department of Anesthesiology, Wake Forest University School of Medicine, Winston-Salem, North Carolina. Submitted for publication September 22, 1998. Accepted for publication June 7, 1999. Supported in part by grant no. GM48085 from the National Institutes of Health, Bethesda, Maryland. Presented in part at the annual meeting of the American Society of Anesthesiologists, Orlando, Florida, October 20, 1988.

Address reprint requests to Dr. Eisenach: Department of Anesthesiology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, North Carolina 27157-1009. Address electronic mail to: eisenach@wfbmc.edu

we chose the latter model because it can be tested in normal human volunteers.

Materials and Methods

All experiments were approved by the Animal Care and Use Committee of Wake Forest University School of Medicine. We did not assess or influence the hormonal cycle of the female rats in this study.

Allodynia Induced by Spinal Nerve Ligation

Male and female Sprague Dawley rats (Harlan, Indianapolis, IN) weighing 150–180 g underwent spinal nerve ligation under halothane anesthesia as previously described.⁷ The left L5 and L6 spinal nerves were isolated adjacent to the vertebral column and tightly ligated with 6-0 silk sutures distal to the dorsal root ganglion. After surgery, animals were housed individually and allowed to recover for at least 2 weeks. Left paw mechanical allodynia was confirmed at this time by evaluation of hind paw withdrawal threshold in response to application of von Frey filaments, using an up-down method previously described.⁹ Only animals with withdrawal thresholds less than 4 g were used. An intrathecal catheter (polyethylene 10 tubing) was inserted as previously described¹⁰ through a small hole in the cisterna magnum and directed caudally such that the tip lay in the intrathecal space around the lumbar enlargement. Animals that showed neurologic deficits were immediately killed by an overdose of pentobarbital; the others were allowed to recover 4–5 days before drug testing. The animals were 10–12 weeks of age at the time of drug testing.

Each group of rats included six males and six females. Testing occurred at the same time during the day. Each animal received a maximum of two agonist injections separated by at least 4 days to avoid development of tolerance. The effect of the following cholinergic agonists to relieve mechanical allodynia in this model was evaluated by intrathecal cumulative dose injections (15-min intervals between doses): neostigmine (cholinesterase inhibitor and indirect agonist), 0.1, 0.3, 0.7, 1, 2, and 5 μ g; bethanechol (nonselective muscarinic agonist), 10, 20, 50, 100, and 200 μ g; RJR-2403 [(E)-N-methyl-4-(3-pyridinyl)-3-butene-1-amine fumarate, a specific neuronal nicotinic agonist acting on $\alpha 4\beta 2$ receptors], 10, 20, 50, and 100 μ g; carbachol (a nonspecific agonist with predominant muscarinic effect), 0.3, 0.7, 1, 2, and 5 μ g. The dose producing a 50% return to presurgery with-

drawal threshold (ED_{50}) was calculated for each drug in male and female rats.

To confirm the sex difference revealed by agonist injections and to assess the role of muscarinic, nicotinic, and adrenergic receptors mediating the antiallodynic effect of neostigmine, rats received a single dose of neostigmine (1 μ g) preceded by pretreatment with saline or one of the following antagonists: 10 μ g atropine (muscarinic antagonist), 10 μ g mecamylamine (nicotinic antagonist), or 30 μ g phentolamine (α -adrenergic antagonist). Antagonist doses were chosen based on reports of blockade of responses to specific agonists in rats.^{11,12}

Allodynia Induced by Intraplantar Capsaicin Injection

Adult male and female Sprague Dawley rats were prepared with insertion of intrathecal catheters as previously described. Capsaicin (*trans*-8-methyl-N-vanillyl-6-nonenamide) was prepared in a concentration of 30 μ g in 10 μ l using the method described by Simone *et al.*¹³ Briefly, capsaicin powder was first dissolved in Tween 80, then in saline isotonic and heated to 70°C. The solution obtained was filtered and stored in sterile glass vials. Each group of rats included six males and six females tested at the same time, and each animal received only one injection in the left plantar surface of the hind paw. Each animal was lightly anesthetized under halothane and immediately after immobility received 30 μ g intradermal capsaicin injected through a 28-gauge needle in the center of the plantar surface. This dose was chosen because it produces mechanical hyperalgesia that lasts at least 2 h.⁸ Recovery from anesthesia was rapid, and all of the animals displayed a nocifensive behavior that lasted < 5 min, then behaved normally and used the paw for locomotion. Hind-paw withdrawal thresholds were tested before capsaicin injection and every 30 min thereafter for 2 h, using von Frey filaments and the up-down method previously described. Care was taken to avoid direct contact of the injection point when measuring withdrawal threshold with the filaments.

Efficacy of spinal cholinergic agonists to prevent development of mechanical allodynia induced by capsaicin was assessed in male and female rats by intrathecal bolus injection of the following drugs before capsaicin injection: saline control, 1 μ g neostigmine, 50 μ g bethanechol, and 50 μ g RJR-2403. In addition, the effect of 10 μ g mecamylamine with 1 μ g neostigmine was investigated. Because of spontaneous pain behavior observed with the combination of atropine and neostigmine and ongo-

SEX DIFFERENCES IN CHOLINERGIC ANALGESIA II

ing pain induced by capsaicin, we did not test an atropine/neostigmine combination in capsaicin studies.

Drugs

The following drugs were used: neostigmine methylsulfate (Gensia, Irvine, CA), bethanechol chloride (Research Biochemicals International, Natick, MA), RJR-2403 (RJ Reynolds Tobacco Co., Winston-Salem, NC), atropine sulfate, mecamlamine, and phentolamine methanesulfonate salt (Sigma, St. Louis, MO). All drugs were dissolved in normal saline and were injected intrathecally in a volume of 5 μ l over 30 s followed by a 10- μ l saline flush.

Statistical Analysis

All data except raw withdrawal threshold (which is expressed in median \pm 25th and 75th percentiles) are presented as mean \pm SE. For raw withdrawal threshold data, effect of drug treatment over time was tested by the Kruskal-Wallis test followed by Dunn's test. For spinal nerve ligation data, withdrawal thresholds after experimental treatment were converted to percent maximum possible effect according to the formula:

$$\text{percent maximum possible effect} = \frac{(\text{postdrug} - \text{presurgery withdrawal threshold})}{(\text{predrug} - \text{presurgery withdrawal threshold})} \times 100$$

All such transformed data were normally distributed. These data were analyzed by one- and two-way analysis of variance followed by Dunnett's test, with $P < 0.05$ considered significant. ED_{50} was calculated by linear regression for each individual animal's dose response. For antagonist studies, the average percent maximum possible effect for 2 h after agonist injection was compared between saline control and antagonist pretreatment groups using one-way analysis of variance.

For capsaicin studies, the effect of antagonists alone were determined by t test. The percent change in threshold was calculated at each observational time compared with the postdrug baseline value. For simplicity, the results are shown as the average reduction in threshold over the 2 h after injection. Within each sex, saline control was compared with drug treatment over time by two-way repeated measures analysis of variance on raw data. Because sexes differed at baseline, sex differences were tested for each drug treatment by two-way repeated measures analysis of variance on the percent change from control over time after capsaicin injection.

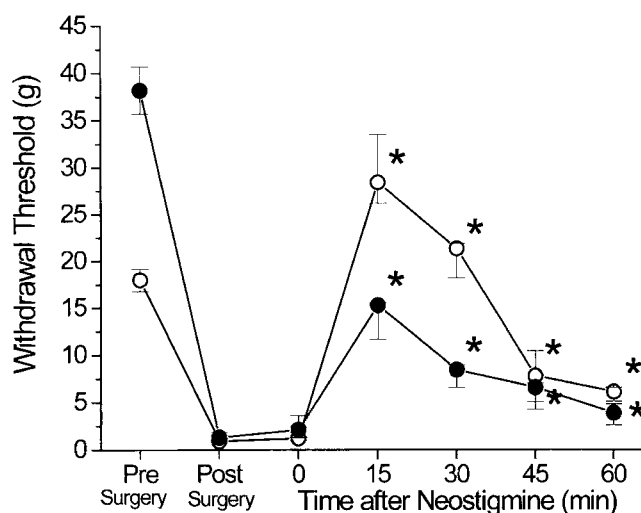


Fig. 1. Withdrawal threshold before and after L5 and L6 spinal nerve ligation in male (closed circles) and female (open circles) rats, and the effect of intrathecal injection of 1 μ g neostigmine in both. Values shown represent the median \pm 25th and 75th percentiles for six animals. Males differ from females before surgery. * $P < 0.05$ compared with postsurgery baseline by Kruskal-Wallis test.

Results

Allodynia Induced by Spinal Nerve Ligation

Before nerve ligation, withdrawal threshold to von Frey filament testing was lower in female than in male animals ($P < 0.01$). Spinal nerve ligation resulted in a decrease in withdrawal threshold within 5 days to a similar level in both sexes, and this allodynia was stable over at least 3 weeks. Intrathecal injection of neostigmine increased withdrawal threshold more in female than in male rats (fig. 1). This was true when data were analyzed as raw withdrawal threshold or percent return to presurgery control.

There were significant sex differences in dose response for antiallodynia for neostigmine, the mixed agonist, carbachol, and the nicotinic agonist RJR-2403 (fig. 2). RJR-2403 showed no significant effect in males. ED_{50} could be calculated for neostigmine and carbachol, and this analysis confirmed increased potency in females compared with males. For neostigmine, ED_{50} was 0.46 ± 0.01 μ g in females compared with 1.3 ± 0.1 μ g in males ($P < 0.05$), and for carbachol, ED_{50} was 0.46 ± 0.01 μ g in females compared with 2.7 ± 0.1 μ g in males ($P < 0.05$). In contrast, there was only a nonsignificant trend ($P = 0.08$) for a sex difference in the response to the muscarinic non-subtype-selective agonist bethanechol (fig. 2). Bethanechol response did not differ at doses $>$

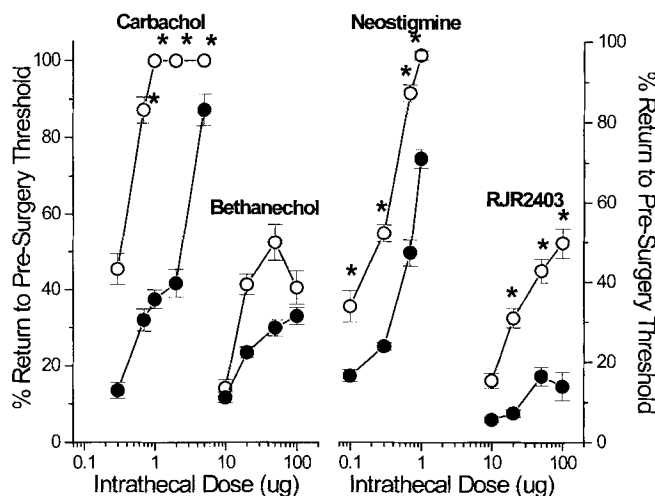


Fig. 2. Dose-dependent analgesic reduction in allodynia expressed as percent return to presurgery threshold after L5 and L6 spinal nerve ligation in male (closed circles) and female (open circles) rats by intrathecal injection of carbachol, bethanechol, neostigmine, and RJR-2403. Values are mean \pm SE for six animals. * $P < 0.05$ compared with males by two-way analysis of variance.

10 μ g in females (e.g., there was an apparent plateau) but not in males (fig. 2).

Intrathecal injection of saline did not alter the antiallodynic effect of neostigmine in either sex (data not shown). In contrast, atropine completely prevented the antiallodynic effect of 1 μ g neostigmine in both sexes (fig. 3). This combination also resulted in spontaneous vocalization and intermittent agitation. There was a sex difference in the effects mecamylamine, which significantly reduced neostigmine's effect in females but not in males. Phentolamine did not alter neostigmine's effect in either males or females (fig. 3). Atropine alone did not affect withdrawal threshold in females (median, 1.8 g before and 1.0 g after atropine) or males (median, 1.6 g before and 0.9 g after atropine). Similarly, mecamylamine alone did not affect withdrawal threshold in females (median, 2.0 g before and 1.1 g after mecamylamine) or males (median, 1.4 g before and 1.9 g after mecamylamine).

Allodynia Induced by Intraplantar Capsaicin Injection

Intraplantar injection of capsaicin produced a rapid-onset, sustained reduction in withdrawal threshold in both sexes (fig. 4). However, male and female control animals differed in withdrawal threshold at baseline and after capsaicin (fig. 4), and the sexes were therefore compared by percent change from precapsaicin threshold. By this analysis, the average percent reduction in

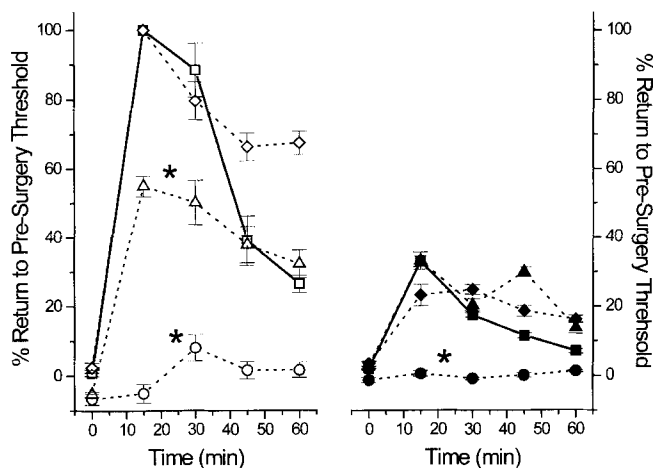


Fig. 3. Antiallodynic effect, expressed as percent return to pre-surgery threshold after L5 and L6 spinal nerve ligation after intrathecal injection of 1 μ g neostigmine at time 0 in females (left, open symbols) and males (right, closed symbols). Fifteen minutes before neostigmine, animals received intrathecal saline (squares), 10 μ g atropine (circles), 10 μ g mecamylamine (triangles), or 30 μ g phentolamine (diamonds). Saline pretreatment is emphasized with the solid line. Values are mean \pm SE for six animals. * $P < 0.05$ compared to saline by two-way analysis of variance.

withdrawal threshold over the 2 h after capsaicin injection was $74 \pm 5.3\%$ in females and $64 \pm 6.7\%$ in males ($P = 0.47$, not significant). Drug pretreatment increased withdrawal threshold significantly only in female rats that received bethanechol (table 1). Intrathecal injection of saline had no effect on capsaicin-induced allodynia in either sex. In contrast, neostigmine diminished allodynia

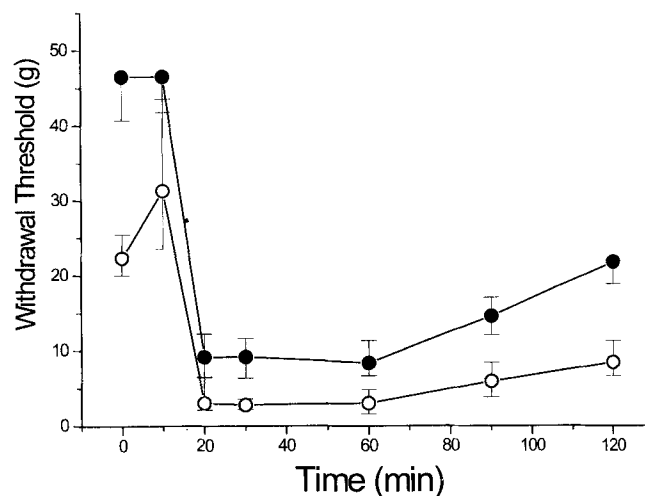


Fig. 4. Withdrawal threshold before and after intraplantar injection of capsaicin at time 15 min in male (closed circles) and female (open circles) rats. Values are expressed as median \pm 25th and 75th percentiles for six animals.

SEX DIFFERENCES IN CHOLINERGIC ANALGESIA II

Table 1. Effects of Drugs on Withdrawal Threshold before Capsaicin Testing

Drug	Male		Female	
	Before	After	Before	After
Saline				
Median	46.5	46.5	22.8	31.3
25th, 75th percentiles	40.7, 46.5	41.8, 46.5	22.2, 24.4	26.3, 37.8
Bethanechol				
Median	58.6	58.6	22.3	58.6*
25th, 75th percentiles	36.5, 58.6	49.6, 58.6	15.6, 22.3	58.6, 58.6
Neostigmine				
Median	27.7	46.5	21.6	46.5
25th, 75th percentiles	21.3, 37.9	40.8, 46.5	20.9, 46.5	46.5, 46.5
Neostigmine plus mecamlamine				
Median	37.9	46.5	18.7	21.6
25th, 75th percentiles	27.1, 46.5	46.5, 46.5	15.4, 22.2	20.9, 26.3
RJR-2403				
Median	22.3	46.8	13.2	22.3
25th, 75th percentiles	22.3, 49.6	25.4, 55.6	11.7, 22.3	22.3, 22.3

* $P < 0.05$ compared with value before drug administration.

from capsaicin to a similar degree in males and females (fig. 5). The nicotinic agonist RJR-2403 reversed capsaicin-induced allodynia to a similar degree in males and females. Bethanechol reversed capsaicin-induced allodynia only in females but also affected withdrawal threshold before capsaicin in this group, making interpretation of this difference difficult. To examine further the role of muscarinic receptors, a combination of neostigmine and mecamlamine was administered. This did not affect withdrawal threshold in either sex before capsaicin (table 1), but significantly more effectively blocked capsaicin-induced hypersensitivity in females than in males (fig. 5).

Discussion

Given the difficulty in the treatment of many patients with neuropathic pain syndromes, which are often considered to be less responsive to opioids,¹⁴ there has been considerable interest in development of novel, nonopioid analgesics for this use. At the same time, multiple animal models of hyperalgesia and allodynia have been described, and the anatomic, physiologic, and molecular changes responsible for their altered sensitivity have been explored. Of particular interest have been models that display allodynia to touch, because this is a common and bothersome feature of patients with neuropathic

pain. We observed efficacy of intrathecal neostigmine in two such models—spinal nerve ligation and intraplantar capsaicin injection—but in only one of these models was neostigmine more potent in female than in male rats. Intrathecal neostigmine produces intense nausea at doses producing analgesia,¹⁵ and, although its use has been anecdotally reported in patients with chronic pain,¹⁶ its usefulness in clinical treatment is uncertain. However, the current descriptive study of the role of spinal cholinergic stimulation in alleviating mechanical allodynia leads to testable hypotheses regarding the pathophysiology of these hypersensitive states and sex differences in these processes and may lead to clinical development of more useful analgesics.

In our companion article in this issue,⁵ we describe an increased sensitivity to the antinociceptive effect of intrathecal neostigmine in normal female compared with male rats. This sex difference is associated with a combined muscarinic and nicotinic component in females but only a muscarinic component in males. The current results support a similar sex difference in the antiallodynic effect of spinal cholinergic agents in the spinal nerve ligation model of chronic pain. Thus, stimulation of spinal muscarinic receptors reduces allodynia in this model in both sexes, as evidenced by similar effects in each sex to the muscarinic agonist bethanechol and reduction in neostigmine's effect by atropine. The nico-

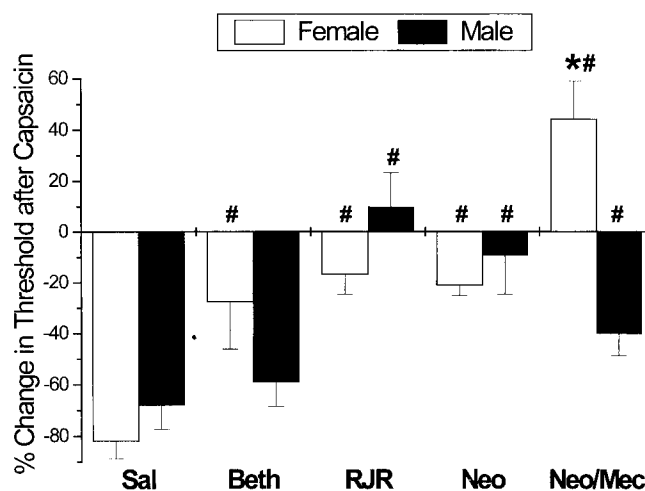


Fig. 5. Average percent change in withdrawal threshold for 2 h after intraplantar injection of capsaicin in female (open bar) and male (closed bar) rats pretreated with intrathecal saline (Sal), 50 μ g bethanechol (Beth), 50 μ g RJR-2403 (RJR), 1 μ g neostigmine (Neo), or 10 μ g Neo plus mecamlamine (Mec). Change is from the withdrawal threshold after drug treatment, but just before capsaicin. Values expressed as mean \pm SE for six animals. * $P < 0.05$ compared with males. # $P < 0.05$ compared with saline control.

tinic component is only present in the female, as evidenced indirectly by increased potency of the mixed agonist carbachol in females and, more directly, by increased potency of the neuronal nicotinic agonist RJR-2403 and by mecamylamine sensitivity of neostigmine's antiallodynic effect only in this sex.

In contrast to our observations in normal animals,⁵ in this nerve injury model, neostigmine's analgesic effect in females was not dependent on spinal noradrenergic stimulation because it was not appreciably diminished by phentolamine. Because α_2 -adrenergic agonists are extremely effective in this model,¹⁷ and because norepinephrine, the endogenous ligand for this receptor, is released from nicotinic receptor stimulation,⁶ we anticipated that increased noradrenergic stimulation after intrathecal neostigmine would be responsible for its increased potency in females. According to the current results, this is not the case. Although the reasons for this change in pharmacology after nerve injury are unclear, these results suggest that nicotinic and α_2 -adrenergic agonists act *via* different mechanisms in the spinal cord to relieve allodynia in this model, and their interaction may therefore be supraadditive.

Intradermal injection of capsaicin results in mechanical allodynia in humans¹³ and rats⁸ and is of interest because this allodynia results from spinal, not peripheral, sensitization¹⁸ dependent on *N*-methyl-D-aspartate receptor activation¹⁹ and can be used for studies in human volunteers of these processes.²⁰ Intrathecal neostigmine reduces allodynia after intraplantar capsaicin injection, but its pharmacology is completely different from that of the nerve injury model. In complete contrast to the results after nerve injury, the antiallodynic effect of intrathecal neostigmine is the same in female and in male rats, as is the antiallodynic effect of the neuronal nicotinic receptor agonist RJR-2403. The muscarinic agonist bethanechol, which shows no sex difference in effect against nerve injury-induced allodynia, is effective only in females after capsaicin injection, although interpretation of this result is complicated by effects of bethanechol before capsaicin injection. The redundant experiment testing the muscarinic component (neostigmine plus mecamylamine) demonstrated a significantly greater effect in females than in males. Although only single doses were studied in these experiments, they were chosen based on their efficacy in other models (including the nerve injury model in the current study) and have been shown to be effective as selective agonists and antagonists at these doses after intrathecal injection in rats. Thus, there was a shared muscarinic

mechanism to neostigmine's action in both sexes to acute noxious stimulation⁵ and to allodynia after nerve injury, whereas there seems to be a shared nicotinic mechanism to neostigmine's action in both sexes to allodynia after capsaicin injection.

The reasons for this difference in pharmacology in the different models are unclear. Spinal nicotinic receptor stimulation results in excitatory amino acid release.²¹ It is conceivable that this acute release may lead to desensitization of *N*-methyl-D-aspartate receptors, which is important in the capsaicin model of acute allodynia is less so in the chronic setting after nerve injury or in response to acute noxious stimulation. It should be noted that the timing of drug testing in the current study differed in the two models of hypersensitivity: pretreatment in the capsaicin model and posttreatment in the nerve injury model. This reflects the different utility of the models, with capsaicin representing an acute central plasticity that can be tested in human volunteers when capsaicin is administered at the time of maximum drug effect, and with nerve injury representing a model of established hypersensitivity. Regardless of the cause, these results suggest that a sex difference in response to spinal cholinergic agents for neuropathic pain may not be appreciated by screening with intradermal capsaicin testing in healthy volunteers.

In summary, intrathecal neostigmine reduces mechanical allodynia in two well-characterized rodent models. Allodynia after nerve injury is reduced by intrathecal neostigmine with a greater potency in females than in males, reflecting a mixture of nicotinic and muscarinic effects in females compared with a purely muscarinic effect in males. In contrast, allodynia after capsaicin is reduced by intrathecal neostigmine with similar potency in females and males, reflecting a mixture of nicotinic and muscarinic effects in females and a nicotinic effect in males. These results suggest that spinal cholinergic receptor stimulation affects allodynia in male and female rats by fundamentally different processes in these two models and suggests caution in extrapolating the results of one model to the other, or from either model to patients with chronic pain.

References

1. Berkley KJ: Sex differences in pain. *Behav Brain Sci* 1997; 20: 371-82
2. Coyle DE, Sehlhorst CS, Mascari C: Female rats are more susceptible to the development of neuropathic pain using the partial sciatic nerve ligation (PSNL) model. *Neurosci Lett* 1995; 186:135-8

SEX DIFFERENCES IN CHOLINERGIC ANALGESIA II

3. Von Korff M, Dworkin SF, Le Resche L, Kruger A: An epidemiologic comparison of pain complaints. *Pain* 1988; 32:173-83
4. Mogil JS, Sternberg WF, Kest B, Marek P, Liebeskind JC: Sex differences in the antagonism of swim stress-induced analgesia: Effects of gonadectomy and estrogen replacement. *Pain* 1993; 53:17-25
5. Chiari A, Tobin JR, Pan HL, Hood DD, Eisenach JC: Sex differences in cholinergic analgesia I: A supplemental nicotinic mechanism in normal females. *ANESTHESIOLOGY* 1999; 91:1447-54
6. Sacaan EI, Menzaghi F, Dunlop JL, Correa LD, Whelan KT, Lloyd GK: Epibatidine: A nicotinic acetylcholine receptor agonist releases monoaminergic neurotransmitters: In vitro and in vivo evidence in rats. *J Pharm Exp Ther* 1996; 276:509-15
7. Kim SH, Chung JM: An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain* 1992; 50:355-63
8. Gilchrist HD, Allard BL, Simone DA: Enhanced withdrawal responses to heat and mechanical stimuli following intraplantar injection of capsaicin in rats. *Pain* 1996; 67:179-88
9. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL: Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* 1994; 53:55-63
10. Yaksh TL, Rudy TA: Chronic catheterization of the spinal subarachnoid space. *Physiol Behav* 1976; 7:1032-6
11. Gillberg PG, Hartvig P, Gordh T, Sottile A, Jansson I, Archer T, Post C: Behavioral effects after intrathecal administration of cholinergic receptor agonists in the rat. *Psychopharmacology (Berlin)* 1990; 100:464-9
12. Aimone LD, Jones SL, Gebhart GF: Stimulation-produced descending inhibition from the periaqueductal gray and nucleus raphe magnus in the rat: Mediation by spinal monoamines but not opioids. *Pain* 1987; 31:123-36
13. Simone DA, Baumann TK, LaMotte RH: Dose-dependent pain and mechanical hyperalgesia in humans after intradermal injection of capsaicin. *Pain* 1989; 38:99-107
14. Arner S, Meyerson BA: Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain. *Pain* 1988; 33:11-23
15. Hood DD, Eisenach JC, Tuttle R: Phase I safety assessment of intrathecal neostigmine in humans. *ANESTHESIOLOGY* 1995; 82:331-43
16. Klamt JG, Dos Reis MP, Neto JB, Prado WA: Analgesic effect of subarachnoid neostigmine in two patients with cancer pain. *Pain* 1996; 66:389-91
17. Yaksh TL, Pogrel JW, Lee YW, Chaplan SR: Reversal of nerve ligation-induced allodynia by spinal alpha-2 adrenoceptor agonists. *J Pharmacol Exp Ther* 1995; 272:207-14
18. LaMotte RH, Lundberg LER, Torebjork HE: Pain, hyperalgesia and activity in nociceptive C units in humans after intradermal injection of capsaicin. *J Physiol* 1992; 448:749-64
19. Dougherty PM, Willis WD: Enhanced responses of spinothalamic tract neurons to excitatory amino acids accompany capsaicin-induced sensitization in the monkey. *J Neurosci* 1992; 12:883-94
20. Park KM, Max MB, Robinovitz E, Gracely RH, Bennett GJ: Effects of intravenous ketamine, alfentanil, or placebo on pain, pinprick hyperalgesia, and allodynia produced by intradermal capsaicin in human subjects. *Pain* 1995; 65:163-72
21. Khan IM, Marsala M, Printz MP, Taylor P, Yaksh TL: Intrathecal nicotinic agonist-elicited release of excitatory amino acids as measured by in vivo spinal microdialysis in rats. *J Pharmacol Exp Ther* 1996; 278:97-106