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Oral Donepezil Reduces Hypersensitivity after Nerve Injury by a Spinal Muscarinic Receptor Mechanism

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Background: **Cholinesterase inhibitors which reach the central nervous system produce pain relief but are poorly tolerated because of gastrointestinal side effects. Here, the authors tested whether donepezil, a central nervous system penetrant cholinesterase inhibitor with a low incidence of gastrointestinal side effects, would relieve hypersensitivity in an animal model of neuropathic pain.**

Methods: **Male rats were anesthetized, and the L5 and L6 spinal nerves were ligated unilaterally. Hypersensitivity was measured by withdrawal threshold to von Frey filament application to the hind paw after oral donepezil, and antagonists administered centrally and peripherally. Efficacy of chronic oral donepezil to relieve hypersensitivity was tested, and activation of G proteins by M_2 muscarinic receptors was determined by carbachol-stimulated [^{35}S]guanosine triphosphate $^{\gamma}$S autoradiography in brain and spinal cord.**

Results: **Spinal nerve ligation resulted in hypersensitivity that was more severe ipsilateral than contralateral to surgery. Oral donepezil reduced hypersensitivity bilaterally in a dose-dependent manner for 2 h, and this effect was blocked by spinal but not supraspinal or peripheral muscarinic receptor antagonism. Oral donepezil maintained efficacy over 2 weeks of twice daily administration, and this treatment did not lead to desensitization of muscarinic receptor–coupled G proteins in brain or spinal cord.**

Conclusions: **Donepezil, a well-tolerated cholinesterase inhibitor used in the treatment of Alzheimer dementia, reduces hypersensitivity in this rat model of neuropathic pain by actions on muscarinic receptors in the spinal cord. Lack of tolerance to this effect, in contrast to rapid tolerance to direct receptor agonists, suggests that cholinesterase inhibition may be useful in the treatment of neuropathic pain.**

NEUROPATHIC pain remains an unmet medical need, in part because of poor efficacy of existing treatments and in part because of their therapy limiting side effects. Opioids, for example, demonstrate acute efficacy in treating neuropathic pain,[1] but their prolonged administration is frequently limited by dose escalation and side effects. For these reasons, alternative treatments have been sought, usually guided by efficacy in rodent peripheral nerve injury models of neuropathic pain.

The current study focuses on cholinergic mechanisms of analgesia. Cholinesterase inhibitors have long been recognized to produce acute analgesia in humans,[2] and animal studies indicate supraspinal,[3] spinal,[4] and peripheral[5] sites of action. Several experiments suggest that cholinergic analgesia is augmented after peripheral nerve injury. As such, peripheral nerve injury results in novel cholinergic circuits that underlie analgesia from spinal adrenoceptor activation[6] and increased expression of cholinergic receptors on sensory afferents,[7] which could produce analgesia when stimulated at their peripheral terminals or those in the spinal cord. One purpose of the current study was to determine the site of action of analgesia from systemic administration of a cholinesterase inhibitor after nerve injury. Because both muscarinic and nicotinic receptors can be involved,[6] we also determined which of these types of cholinergic receptors were important to cholinesterase inhibitor analgesia.

Although intravenous physostigmine[8] and intrathecal neostigmine[9] produce analgesia in humans, they are not used clinically, because of commonly occurring and severe nausea. The current study examined an orally active cholinesterase inhibitor, donepezil (Aricept; Pfizer, New York, NY), currently approved for the treatment of Alzheimer dementia. Donepezil is concentrated in the central nervous system after oral administration[10] and is well tolerated in the elderly, with a low ($< 5\%$) incidence of therapy-limiting tolerance.[11] Rats do not show nausea, so we could not determine the therapeutic ratio of donepezil in this species, but we tested the range of oral doses in rats which produce cholinesterase inhibition of similar degree to therapeutic doses in humans,[10,11] and determined its efficacy to reduce hypersensitivity to mechanical stimulation in a peripheral nerve injury model of neuropathic pain.[12]

Cholinesterase inhibitors reduce sedation and respiratory depression from opioids.[8] A recent clinical study demonstrated a reduction in opioid-induced sedation in cancer patients when donepezil was added, but a secondary analysis suggested that this beneficial effect might be reduced with chronic dosing.[13] Tolerance to opioid, α_2-adrenoceptor, and cholinergic agonists develops rapidly in rats[14,15] and leads to receptor desensitization as determined by a decrease in the ability of agonists to these receptors to stimulate [^{35}S]guanosine triphosphate (GTP) $^{\gamma}$S binding to G proteins.[16] Whether tolerance similarly occurs when availability of the endogenous agonist is increased, such as acetylcholine after cholinesterase inhibition, is uncertain, and a final pur-

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pose of the current study was to determine whether chronic donepezil maintained efficacy in reducing hypersensitivity *in vivo* and produced receptor/G-protein desensitization *in vitro*.

Materials and Methods

Animals, Surgery, and Behavioral Testing

Animal procedures adhered to the Wake Forest University guidelines on the ethical treatment of animals and were approved by the Animal Care and Use Committee, Wake Forest University School of Medicine, Winston-Salem, North Carolina. Male Sprague-Dawley rats (Harlan, Industries, Indianapolis, IN), weighing 125–150 g were used in the current study. Animals were housed with a 12-h dark–light cycle with water and food *ad libitum*.

All animals underwent spinal nerve ligation (SNL) surgery as previously described.[12] Anesthesia was induced and maintained with halothane in oxygen and spontaneous ventilation. The lateral laminae of both the lower lumbar and upper sacral vertebrae were exposed. After the right transverse L6 process was removed, both spinal nerve roots L5 and L6 were identified and ligated by means of a silk 6-0 suture. Before closing the wound, antibiotic ointment was placed in the incision, and rats received 2 ml subcutaneous penicillin–streptomycin.

Sensitivity to light touch was tested by means of von Frey filaments applied to the plantar region of the hind paws (Stoelting, Wood Dale, IL) 2 weeks after SNL surgery. Animals were placed on mesh flooring in clear plastic partitions and allowed to acclimate before testing. A filament was applied to the plantar surface of the paw with enough force to bend it, and a brisk paw withdrawal was considered a positive response. Withdrawal threshold was measured using an up–down statistical method.[17] Only animals without signs of motor weakness and with withdrawal threshold after SNL surgery of 4 g or less were studied.

Intrathecal catheters were inserted in some animals 2 weeks after SNL as previously described.[18] During halothane anesthesia, the head was placed in a stereotaxic frame and a small incision was made at the base of the neck. A polyethylene catheter, 8.5 cm, was introduced through a slit in the atlanto-occipital membrane of the cisterna magnum. The catheter was inserted until the caudal tip reached the lumbar enlargement of the spinal cord and the rostral end was exteriorized at the head. The wound was sutured, and animals were thereafter caged individually. Animals with motor weakness or paralysis after surgery were to be immediately killed, but this was not observed or needed in the current study. Animals were allowed 5 days to recover before behavioral studies.

Intracerebroventricular cannulae were inserted in some animals 2 weeks after SNL. During pentobarbital anesthesia (50 mg/kg, intraperitoneally), the head was placed in a stereotaxic frame and a small incision was made to expose the skull. The skull surface was cleaned before the implantation of the stainless steel cannula. The coordinates for placement of the tip of the guide cannula were 0.80 mm posterior and 1.5 mm lateral to the bregma, and 3.5 mm ventral from the surface of the dura mater, according to the rat brain atlas.[19] The cannula was secured using dental resin along with a self-tapping screw. Before closing the wound, the guide cannula was capped with a dummy cannula, and 2 ml subcutaneous penicillin–streptomycin was administered. Animals recovered over a period of 5 days and were used only if there was no motor impairment after surgery.

Nociceptive withdrawal threshold to paw pressure was determined in normal animals, using the Randall-Selitto test and an analgesimeter (Ugo Basile, Comerio, Italy). The animal was gently restrained and the hind paw was placed between a clear plastic surface and a plinth. By pressing a pedal that activated a motor, the force applied to the plinth increased at a constant rate on the linear scale. When the animal withdrew the paw or vocalized, the pedal was immediately released and the nociceptive threshold was read on a scale. A cutoff of 250 g was used to avoid potential tissue injury.

Acute Drug Administration

Oral administration of donepezil and vehicle was performed in a volume of 5 ml/kg through an orogastric feeding tube (Solomon Scientific, Plymouth Meeting, PA). Donepezil (Aricept) pills were crushed to a fine powder using a mortar and pestle then dissolved in 0.5% carboxymethylcellulose solution. To determine dose response, eight animals received donepezil 2.5 mg/kg, 10 mg/kg, and vehicle, with experiments separated by 4–5 days. Withdrawal threshold was determined before drug administration and at 30 min and 1, 2, and 4 h thereafter. The experimenter was blind to treatment group.

Several studies were performed with antagonists to determine the pharmacologic mechanism and site of action of donepezil. Drug doses and timing were determined in unblinded pilot experiments, followed by blinded, randomized studies with seven animals per group. The muscarinic antagonist atropine was administered subcutaneously (0.03–1 mg/kg) in a randomized manner simultaneously with oral donepezil, and withdrawal threshold was determined 1 h later. To test a spinal site of action, animals received intrathecal vehicle or atropine (3 and 30 μg in 5 μl with a 10-μl saline flush) 30 min after oral donepezil, with withdrawal threshold measured 30 min later (1 h after donepezil). To test a supraspinal site of action, animals received intracerebroventricular injection of vehicle or atropine, 3 μg in 5 μl 45 min after oral donepezil, with withdrawal threshold measured 15 min later (1 h after donepezil). We also

examined the effect of intracerebroventricular atropine after oral vehicle, using the same dose and timing. To test a peripheral site of action, animals received a subcutaneous injection of atropine methyl nitrate, a peripherally restricted muscarinic antagonist, 2–5 mg/kg simultaneous with donepezil, and withdrawal threshold was measured 1 h later.

Because the above studies demonstrated a primary spinal site of action, we also tested whether donepezil relied on spinal nicotinic as well as muscarinic cholinergic receptor activation. In these studies, animals were randomly assigned to receive intrathecal saline, the nicotinic antagonist mecamylamine, 50 μg, or a combination of this dose of mecamylamine and atropine, 3 μg, 30 min after oral donepezil, and withdrawal threshold was determined 30 min later (1 h after donepezil).

Chronic Drug Administration

To test efficacy over time, animals were randomly assigned to receive oral vehicle or donepezil, 10 mg/kg, twice a day, for 2 weeks (n = 6/group). Withdrawal threshold was determined before and at 3, 7, 10, and 14 days after beginning oral treatments by a separate investigator from the one who administered study drug. The investigator performing the behavioral studies was blinded to treatment group.

In Vitro *Autoradiography of Muscarinic Receptor–stimulated [^{35}S]GTPγS Binding in Brain and Spinal Cord Sections*

For [^{35}S]GTPγS binding in sections, brains and spinal cords were frozen in isopentane at −35° and stored at −80°. Coronal sections (20 μm) were cut on a cryostat at −20°C, mounted on gelatin-subbed slides and stored at −80°. Sections were rinsed in assay buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 0.2 mM EGTA, 100 mM NaCl, pH 7.4) at 25° for 10 min, followed by a 15-min preincubation in assay buffer containing 2 mM GDP and 10 mU/ml adenosine deaminase at 25°. Sections were then incubated in assay buffer with 2 mM GDP, 10 mU/ml adenosine deaminase, and 0.04 nM [^{35}S]GTPγS, with (stimulated) or without (basal) 100 μM carbachol at 25° for 2 h. Slides were rinsed twice in cold Tris buffer (50 mM Tris-HCl, pH 7.0) for 2 min and once in deionized water for 30 s.[20] After drying at room temperature overnight, sections were exposed to phosphoimaging screens for 18 h in cassettes containing [^{14}C] standards for densitometric analysis. Phosphoimages were analyzed densitometrically. Values were corrected for [^{35}S] based on incorporation of [^{35}S] into sections of frozen brain paste as previously described,[20] and correction factors were used to convert [^{14}C] values to [^{35}S] data. Net carbachol-stimulated [^{35}S]GTPγS binding was calculated by subtracting basal binding from carbachol-stimulated binding. Data were expressed as nCi [^{35}S]GTPγS/g tissue and are reported as mean values ± SE of triplicate sections of brains from at least six animals.

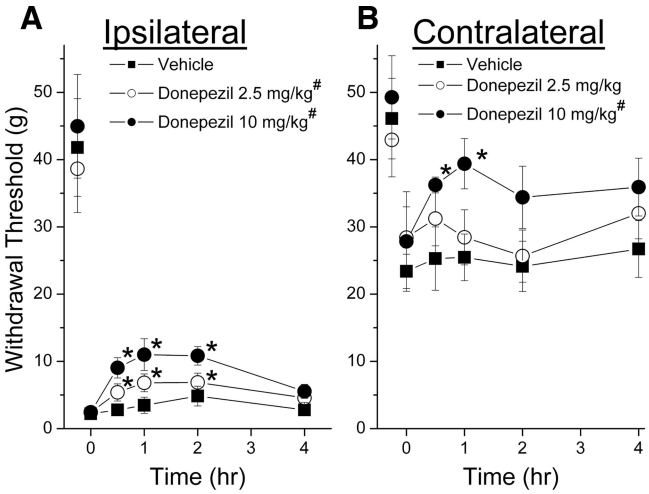

Fig. 1. Effect of oral donepezil on withdrawal threshold to von Frey filament application to the hind paw ipsilateral (*A*) and contralateral (*B*) to spinal nerve ligation. *Symbols to the far left of each panel* represent withdrawal threshold before spinal nerve ligation. Each *symbol* represents the mean ± SE of eight animals. * $P < 0.05$ compared with time 0 by one-way analysis of variance. # $P < 0.05$ compared with vehicle by two-way analysis of variance.

Data Analysis

Data are presented as mean ± SEM and were analyzed by one- and two-way analysis of variance. $P < 0.05$ was considered significant.

Results

Spinal nerve ligation surgery resulted in reduced withdrawal threshold which was more pronounced ipsilateral to surgery, from a population mean of 29 ± 2.5 g before surgery to 1.8 ± 0.2 g ipsilateral to surgery and 22 ± 2.1 g contralateral to surgery (both $P < 0.01$).

Dose Response

Oral donepezil increased withdrawal threshold ipsilateral to surgery in a dose-related manner (fig. 1A; $F_{8,80.06} = 2.5$, $P = 0.018$). Greatest effects were seen 1–2 h after drug administration, with the 10-mg/kg dose significantly different from vehicle and the 2.5-mg/kg dose at both 1- and 2-h time points. Effects on contralateral hypersensitivity were also present and dose related. In this case, the lower dose, 2.5 mg/kg, did not separate from vehicle, but 10 mg/kg did increase withdrawal threshold compared with vehicle (fig. 1B; $F_{2,32.92} = 4.76$, $P = 0.015$). The contralateral effect of donepezil also peaked between 1 and 2 h.

Site and Mechanism of Action

Systemic (subcutaneous) atropine dose dependently and completely inhibited the effects of donepezil on

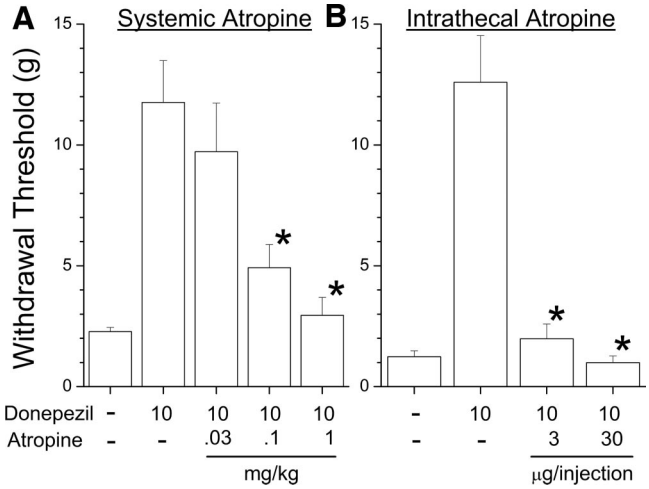

Fig. 2. Effect of systemic (*A*) or intrathecal (*B*) atropine on withdrawal threshold to von Frey filament application to the hind paw ipsilateral to spinal nerve ligation. Dose of donepezil is shown in mg/kg. Each *bar* represents the mean ± SE of six to eight animals. * $P < 0.05$ compared with donepezil, 10 mg/kg alone.

withdrawal threshold ipsilateral to surgery (fig. 2A; $F_{7,70.4} = 3.04$, $P = 0.008$). Intrathecal atropine, which typically is administered in 10- to 30-μg doses intrathecally to rats,[6,21–23] abolished the effect of donepezil in a dose as small as 3 μg (fig. 2B; $F_{3,12.68} = 33.37$, $P < 0.001$).

In contrast to efficacy from systemically administered atropine, systemic administration of the poorly central nervous system–penetrant atropine congener, atropine methyl nitrate did not block the antihypersensitivity effect of donepezil, even in doses up to 5 mg/kg (fig. 3A). Finally, intracerebroventricular administration of atropine, to test supraspinal central nervous system sites of action, did not block the antihypersensitivity effect of donepezil (fig. 3B). Intracerebroventricular injection of atropine alone had no effect on withdrawal threshold (fig. 3B).

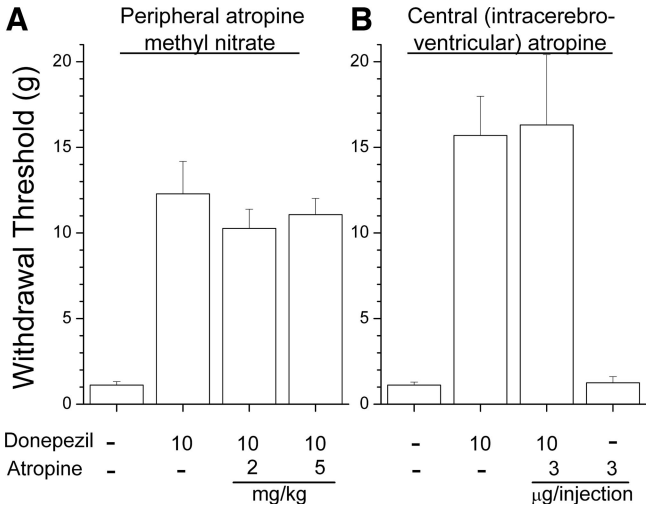

Fig. 3. Effect of systemic administration of the poorly central nervous system penetrant drug, atropine methyl nitrate (*A*) or intracerebroventricular administration of atropine (*B*) on withdrawal threshold to von Frey filament application to the hind paw ipsilateral to spinal nerve ligation. Dose of donepezil is shown in mg/kg. Each *bar* represents the mean ± SE of six to eight animals. No effect of antagonist against donepezil in either case.

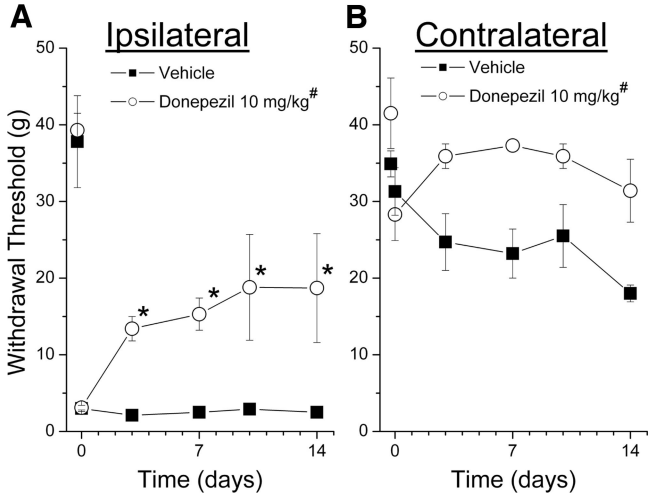

Fig. 4. Effect of oral donepezil, 10 mg/kg twice daily, on withdrawal threshold to von Frey filament application to the hind paw ipsilateral (*A*) and contralateral (*B*) to spinal nerve ligation. *Symbols to the far left of each panel* represent withdrawal threshold before spinal nerve ligation. Each *symbol* represents the mean ± SE of six animals. * $P < 0.05$ compared with time 0 by one-way analysis of variance. # $P < 0.05$ compared with vehicle by two-way analysis of variance.

Intrathecal mecamylamine also did not block the antihypersensitivity effect of oral donepezil. In these animals, withdrawal threshold of the paw ipsilateral to surgery was 1.0 ± 0.1 g without difference between groups. Oral donepezil in the presence of intrathecal mecamylamine significantly increased withdrawal threshold to 12 ± 3.3 g ($P < 0.001$), whereas donepezil did not increase withdrawal threshold in the presence of the combination of intrathecal mecamylamine and atropine (0.6 ± 0.02 g).

Paw pressure was administered to test sensitivity to mechanical stimulation in normal rats, because this represents a noxious stimulus in the normal condition, in contrast to von Frey filament testing.[24] Withdrawal threshold 1 h after oral donepezil, 10 mg/kg (170 ± 10 g), was not increased compared with baseline (167 ± 8 g), demonstrating lack of acute antinociception from this treatment in the absence of nerve injury.

Chronic Drug Administration

Animals appeared normal during the 2 weeks of vehicle or donepezil, 10 mg/kg twice daily, with normal grooming and ambulation and response to handling. Withdrawal threshold ipsilateral to surgery increased in donepezil- but not vehicle-treated animals, and this effect was sustained for the 14 days of treatment (fig. 4A). As in the acute dose–response study, there was a significant effect of treatment on withdrawal threshold con-

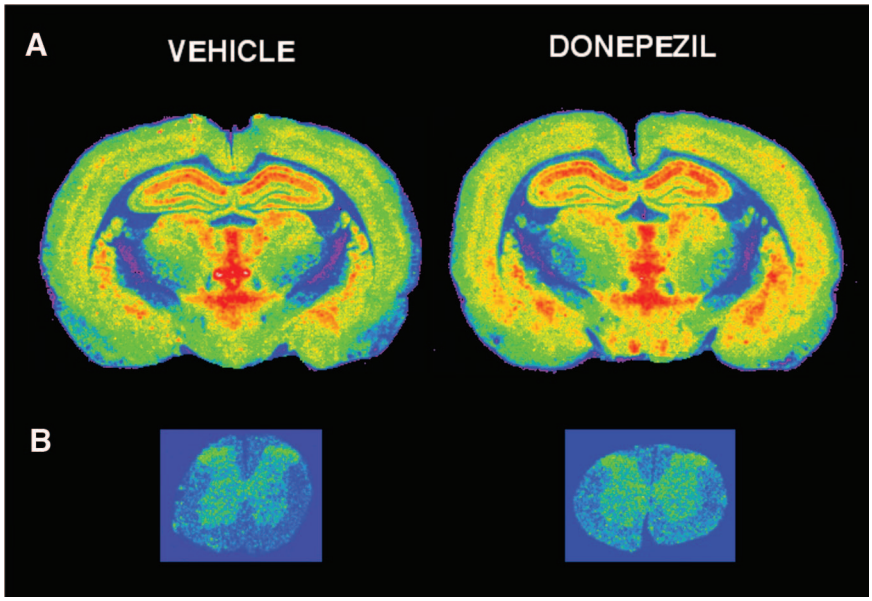

Fig. 5. Autoradiography of muscarinic-stimulated [^{35}S]guanosine triphosphate γS binding in sections from midbrain (*A*) and spinal cord (*B*). *Left*, sections from vehicle-treated rats; *right*, sections from rats treated chronically with donepezil as described in the Materials and Methods. Shown are typical sections incubated *in vitro* with 100 μM carbachol.

tralateral to surgery over the 2-week trial, although the level of hypersensitivity and the drug effect were less than ipsilateral to surgery (fig. 4B).

To determine whether chronic treatment with donepezil had any effect on muscarinic receptor coupling to G proteins, sections from both brain and spinal cord from vehicle- and donepezil-treated rats were assayed for carbachol-stimulated [^{35}S]GTPγS binding. Figure 5 shows representative autoradiograms from both groups of animals. In spinal cord, carbachol stimulated [^{35}S]GTPγS binding in the superficial laminae of dorsal horn, as previously described,[25] whereas in midbrain, significant carbachol-stimulated [^{35}S]GTPγS binding was observed in both thalamus and hippocampus. Chronic treatment of rats with donepezil had no discernible effect on carbachol-stimulated [^{35}S]GTPγS binding in any of these tissues. Densitometric analysis of [^{35}S]GTPγS autoradiography (table 1) confirmed these findings, with no significant change in net carbachol-stimulated binding in dorsal horn, thalamus, or hippocampus after chronic donepezil administration.

Table 1. Effect of Chronic Donepezil Treatment on Muscarinic Receptor–stimulated [^{35}S]GTPγS Binding in Brain and Spinal Cord

	Net Carbachol-stimulated Binding, nCi/g (% of Control)	
Tissue	Vehicle	Donepezil
Spinal cord	40 ± 3 (100 ± 7)	40 ± 3 (101 ± 7)
Thalamus	380 ± 8 (100 ± 2)	400 ± 6 (105 ± 2)
Hippocampus	300 ± 7 (100 ± 2)	300 ± 11 (102 ± 4)

Sections from spinal cord and brain were removed from animals chronically treated with vehicle or donepezil, and assayed for [^{35}S]guanosine triphosphate (GTP) γS autoradiography in the presence and absence of 100 μM carbachol as described in the Materials and Methods. Results are mean values ± SEM of nCi/g [^{35}S] as determined by densitometry (with percent of control in parentheses).

Discussion

The current study provides new mechanistic and practical insights into cholinergic analgesia in the setting of neuropathic pain. From a mechanistic standpoint, the current study demonstrates efficacy of cholinesterase inhibition to reduce hypersensitivity both ipsilateral to injury and its spread contralaterally (so-called mirror image pain). In addition, this study describes the site of action and cholinergic receptor type involved in the antihypersensitivity effect of donepezil and suggests that this drug, unlike direct agonists, may have sustained efficacy to treat pain without dose escalation from tolerance. From a practical standpoint, although other cholinesterase inhibitors have previously been demonstrated to reduce hypersensitivity after nerve injury in animals, this is the first study to examine a clinically available and well-tolerated cholinesterase inhibitor. The observed efficacy from this agent supports its introduction into clinical trials.

Peripheral nerve injury, whether by SNL or acute neuritis of the sciatic nerve, results in hypersensitivity to tactile stimulation bilaterally, although the effect is much more marked on the side of injury.[12,26] This extension of hypersensitivity depends on afferent input from the injured area and may reflect bilateral glial activation in the spinal cord *via* gap junction connections.[27] It is conceivable that donepezil reduced hypersensitivity bilaterally due to actions on glia, but this is unlikely because the immunosuppressive effects of acetylcholine depend primarily on nicotinic receptor activation,[28] and yet the nicotinic receptor antagonist, mecamylamine, was ineffective to block the antihypersensitivity effect of donepezil. More likely, increased acetylcholine in the spinal cord ipsilateral to nerve injury after donepezil reduced afferent input by a presynaptic mechanism[29] or by re-

ducing interneuron activation by a postsynaptic mechanism.[30]

Analgesia from cholinergic agonists has been suggested to reflect actions in the brain,[3] the spinal cord,[4] and at peripheral terminals of sensory afferents.[5] Both muscarinic and nicotinic receptor activation can result in analgesia.[6] Analgesia has also been demonstrated in humans receiving cholinesterase inhibitors systemically with high (physostigmine)[31] or low (neostigmine)[2] ability to penetrate the central nervous system, as well as intrathecal neostigmine,[9] consistent with actions in the brain, spinal cord, or periphery. The current study argues strongly for a spinal site of action on muscarinic receptors to reduce hypersensitivity in nerve-injured rats receiving oral donepezil, because the effect of donepezil was blocked by spinal atropine, but not systemic atropine methyl nitrate or intracerebroventricular atropine in doses of known specificity to block peripheral and supraspinal muscarinic receptors.[32,33] It is unlikely that intrathecal atropine produced a pronociceptive effect that merely counterbalanced the effect of donepezil, because atropine alone has no effect in normal or spinal nerve–ligated animals.[34] Because intrathecal neostigmine produces analgesia in humans, including those with neuropathic pain,[35] it is possible that a spinal site of action of donepezil could be applicable to humans as well.

Tolerance to opioid analgesics is a well-described phenomenon in humans as well as animals and leads to a reduction in efficacy of a fixed dose and shift in the dose response, necessitating dose escalation over time. This phenomenon occurs rapidly in rats, with maximally analgesic doses of opioids totally lacking efficacy after 3–5 days of continuous treatment.[15] A similar speed and degree of tolerance appears with intrathecal injection of the cholinergic agonist, carbachol,[14] but the current study showed no such tolerance with maximally effective doses of donepezil over 2 weeks. Dose escalation is rarely needed with drugs that inhibit reuptake of endogenous neurotransmitters, such as antidepressants, and with cholinesterase inhibitors for the treatment of dementia or myasthenia gravis, perhaps because of differing efficacy of endogenous neurotransmitters to induce receptor desensitization mechanisms than synthetic ligands.

Tolerance to chronic drug administration is often accompanied by a desensitization of receptor-coupled G proteins, as determined by a decrease in agonist-stimulated [^{35}S]GTP$^{\gamma}$S binding. Such desensitization effects have been observed in brain and spinal cord after chronic administration of opioids,[25] Δ^9-tetrahydrocannibinol,[36] and buspirone.[37] The finding in the current study that chronic treatment with donepezil had no effect on muscarinic activation of G proteins is consistent with the lack of tolerance observed in hypersensitivity tests in these same animals. It is possible that elevation of endogenous neurotransmitters by drugs such as donepezil may have differing ability to produce desensitization compared with direct-acting agonists; future studies in our laboratory will examine this issue by directly comparing the effects of chronic donepezil and carbachol on desensitization of muscarinic receptor–coupled G proteins. However, we do know that elevation of endogenous neurotransmitters can produce desensitization of receptor-coupled G proteins in other systems. Recent studies in our laboratory[38] revealed that chronic administration in rats with a potent inhibitor of monoamine transporters produced significant desensitization of dopamine D_2, 5-hydroxytryptamine$_{1A}$, and α_2-adrenergic receptor–stimulated [^{35}S]GTP$^{\gamma}$S binding in several brain areas.[39] However, this transporter blocker had extraordinary affinity at monoamine transporters, with corresponding long duration of action.[40] Therefore, it is possible that the efficacy of such drugs to produce desensitization after chronic administration may depend on their affinity and duration of action.

The ultimate utility of donepezil in treating chronic pain in humans cannot be addressed by studies in animals. The effect of donepezil on hypersensitivity, although significant, was modest compared with that of morphine and gabapentin.[41,42] Intrathecal neostigmine or systemic physostigmine produce severe nausea,[8,9] but therapy with donepezil is limited by nausea in less than 5% of cases.[11] The only study to examine donepezil in patients with pain was designed to test the hypothesis that donepezil reduced sedation from opioids, which it did.[13] Pain scores were numerically reduced in the 19 subjects in that study in whom visual analog pain scores were obtained, from 47 mm before donepezil to 37 mm afterward (0- to 100-mm scale), although this difference was not significant ($P = 0.07$).[13] Based on these encouraging data, we are currently examining efficacy of oral donepezil to treat pain and improve cognition in patients with chronic pain.

Conclusion

In summary, oral donepezil reduces hypersensitivity both ipsilateral and contralateral to SNL in a rat model of neuropathic pain. Inhibition of donepezil by intrathecal but not intracerebroventricular atropine, and by systemic atropine but not atropine methyl nitrate, suggest that oral donepezil reduces hypersensitivity by an action in the spinal cord. Donepezil maintained undiminished efficacy with twice daily dosing for 2 weeks, unlike previous studies with opioid or cholinergic agonists, consistent with a lack of tolerance development. Although we could not assess the incidence of nausea, the most common side effect in humans from this class of drugs, these data in rats strongly support examination of this agent and other cholinesterase inhibitors developed

to treat dementia as analgesics for patients in neuropathic pain.

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