Antitussive Effects of Memantine in Guinea Pigs

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Background: The treatment of cough is a significant clinical unmet need because there is little evidence that current therapies are effective. Based on evidence supporting a role for N-methyl D-aspartate receptors (NMDARs) in cough, we hypothesized that memantine, a low-affinity, uncompetitive NMDAR channel blocker in routine use for the treatment of Alzheimer disease, could be an effective, well-tolerated, antitussive therapy. The aim of this study was to establish preclinical evidence that memantine has antitussive effects.

Methods: We studied the influence of memantine on experimentally induced coughing in response to citric acid and bradykinin inhalation in guinea pigs. We also compared the potency and efficacy of memantine as an antitussive to other NMDAR antagonists, dextromethorphan and ketamine, and to the γ -aminobutyric acid class B receptor agonist baclofen.

Results: Compared with control subjects, 10 mg/kg memantine significantly reduced the cumulative number of coughs evoked by both citric acid (median, 24.0 [interquartile range (IQR), 13.0-25.5] vs 1.5 [IQR, 0.3-10.3] coughs; $P = .012$) and bradykinin aerosols (median, 16.0 [IQR, 9.5-18.5] vs 0.0 [IQR, 0-0.75] coughs; $P = .002$). Memantine 10 mg/kg produced a similar reduction in the cumulative number of coughs to baclofen 3 mg/kg and demonstrated comparatively greater cough suppression than 30 mg/kg dextromethorphan or 30 mg/kg ketamine. This dose of memantine produced no sedative or respiratory depressive effects.

Conclusions: This study illustrates that memantine has marked antitussive effects in guinea pigs, most likely mediated through NMDAR channel blockade. Memantine, therefore, has the potential to be a safe, effective, and well-tolerated antitussive agent. *CHEST* 2012; 141(4):996-1002

Abbreviations: 2M5HT = 2-methyl 5-hydroxytryptamine; IQR = interquartile range; NMDAR = N-methyl D-aspartate receptor; nTS = nucleus tractus solitarius; PCR = polymerase chain reaction

Effective treatments for cough are a significant clinical unmet need. There is little evidence that current therapies are effective, and many are associated with significant side effects. The N-methyl D-aspartate receptor (NMDAR) blocker dextromethorphan has

been used as an antitussive agent for decades and frequently is a component of over-the-counter cough remedies. Dextromethorphan is a low-affinity, uncompetitive NMDAR channel blocker¹ but is also a sigma-1 receptor agonist^{2,3} and voltage-gated calcium channel blocker⁴ and has antitussive effects that translate from animal models to human studies.⁵⁻⁹ However, in the only study to objectively quantify the effect of dextromethorphan compared with placebo in subjects with acute cough, the impact on cough frequency was modest, with a reduction in cough frequency of just 12%.⁵ Concerns about the safety of dextromethorphan and other over-the-counter cough medications,

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especially in children, has led to restrictions in their use.^{10,11}

NMDARs play many diverse roles in the CNS, including synaptic transmission, synaptic plasticity, and neuronal protection and survival. NMDARs are glutamate-gated ion channels that consist of four subunits, typically two NR1 subunits and two NR2 subunits, surrounding a central channel pore. The NR1 subunits are obligatory for functionality and can combine with four different NR2 (A-D) and two different NR3 (A and B) subunits. Subunit expression varies during development and with location. In the inactive state, the channel pore is blocked by Mg²⁺. Partial membrane depolarization is sufficient to relieve this blockade, allowing the influx of Na²⁺ and Ca²⁺. NMDARs possess multiple extracellular binding sites, allowing a variety of molecules to modulate their function.

Like dextromethorphan, memantine (used clinically to treat moderate to severe Alzheimer disease) is a low-affinity, uncompetitive NMDAR blocker, binding preferentially to open NMDAR channels.^{12,13} Memantine, therefore, only blocks activated receptors, providing higher levels of blockade in the presence of high concentrations of glutamate and relatively lower levels of blockade during normal physiologic transmission. This mode of action may explain why memantine treatment is well tolerated by patients. A recent review suggested adverse effects in <10% of patients treated for dementia.¹⁴ In addition to blocking NMDAR channels, memantine may block type 3 serotonin and nicotinic acetylcholine receptor channels at similar concentrations.^{15,16}

Based on the available evidence supporting a role for NMDARs in cough, we hypothesized that memantine may be a well-tolerated antitussive therapy. The aims of this study were to establish preclinical evidence that memantine has antitussive effects on experimentally induced coughing in guinea pigs. We also compared the potency and efficacy of memantine as an antitussive to that of the NMDAR blockers dextromethorphan and ketamine as well as the γ -aminobutyric acid class B receptor agonist baclofen.

MATERIALS AND METHODS

Animals

Male Hartley guinea pigs (200-700 g) (Hilltop Lab Animals, Inc) were studied. All experiments were first approved by the institutional Animal Care and Use Committee.

Citric Acid-Induced Cough

Animals were placed in a transparent chamber (Buxco Research Systems) with a continuous flow of air and exposed to increasing concentrations of citric acid (0.01-0.3 mol/L) delivered by an

ultrasonic nebulizer generating aerosol particles of 3 to 6 μ m in diameter. Coughs were counted during a 5-min nebulization period and over the subsequent 5 min with the assistance of sound and pressure monitoring from the chamber. Respiratory rate and tidal volume were monitored throughout with a calibrated pressure transducer (Emka Technologies).

Bradykinin-Induced Cough

Using a similar chamber and nebulizer system, animals were treated first with aerosolized peptidase inhibitors (captopril 0.1 μ mol/L and thiorphan 0.1 μ mol/L, 5 min nebulization) to reduce bradykinin degradation and enhance tussive responses evoked by bradykinin (data not shown). Animals were then exposed to increasing concentrations of aerosolized bradykinin (0.1-3 mg/mL), again for 5-min periods. Coughs were counted during this and the subsequent 5 min. Pressure changes within the chamber were used to monitor respiratory rate (Biopac Systems Inc).

Responses to IV 2-Methyl 5-Hydroxytryptamine and Mecamylamine

Animals were anesthetized (1.5 g/kg intraperitoneal urethane) and cannulae placed in the carotid artery and jugular vein to monitor arterial pressure and to administer drugs, respectively. A cannula was also placed in the extrathoracic trachea, through which animals spontaneously breathed warmed, moistened air while ventilatory pressures were monitored through a side-port. Arterial and airway pressures were recorded digitally (Biopac Systems). Animals were treated with either intraperitoneal memantine (10 mg/kg) or vehicle (0.9% saline) given 30 min before the administration of 2-methyl 5-hydroxytryptamine (2M5HT) and mecamylamine. IV bolus doses of 2M5HT (10 and 100 μ g/kg) followed by mecamylamine (1 and 3 mg/kg) were given at 5-min intervals.

NMDAR Subunit Expression

Animals were killed with phenobarbital (150 mg/kg) and tissue harvested from the nodose and jugular ganglia, cerebellum, and lung parenchyma and by punch biopsy taken from the nucleus tractus solitarius (nTS). Tissues were then frozen in RNAlater (Qiagen Inc) prior to RNA isolation using the RNeasy Plus Mini Kit (Qiagen Inc) according to the manufacturer's instructions. Synthesis of cDNA was performed using Omiscript Reverse Transcriptase (Qiagen Inc). The resulting cDNA product was then used to carry out polymerase chain reactions (PCRs). After an initial activation at 95°C for 15 min, cDNAs were amplified with custom-synthesized primers (Invitrogen Corporation) (e-Table 1) by 50 cycles, denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min followed by a final extension at 70°C for 10 min. Products were then visualized in ethidium bromide-stained 1.5% agarose gels.

Compounds and Materials

Memantine, citric acid, bradykinin, mecamylamine, captopril, thiorphan, ondansetron, and baclofen were supplied by Sigma-Aldrich Co LLC; dextromethorphan by MP Biomedicals, LLC; 2M5HT by Tocris Bioscience; and ketamine by Vedco Inc. All drugs were dissolved in 0.9% saline except citric acid (dissolved in distilled water) and thiorphan (dissolved in ethanol and then diluted in 0.9% saline). Memantine, ketamine, baclofen, ondansetron, and placebo were administered by intraperitoneal injection 30 min prior to cough challenges; the experimenters were not blinded to the agent identity. Dosing and administration were based on studies in rats where memantine half-life is 2.5 h at

10 mg/kg¹⁷ and receptor occupancy approximately one-third at doses of 12.5 mg/kg per day.¹⁸ Any sedation caused by these agents was assessed subjectively and classified as severe if animals were unable to stand, moderate if unstable when walking, and mild if just showing reduced spontaneous movement.

Statistical Analysis

Data were analyzed using SPSS, version 15 (SPSS Inc), and graphs were produced using Prism, version 4 (GraphPad Software Inc). A 5% level of significance was used throughout. Cumulative numbers of coughs to citric acid and bradykinin challenges were expressed as median and interquartile range (IQR) because the data were positively skewed. Comparisons of cumulative cough numbers for treatment groups, therefore, were compared using nonparametric tests (Kruskal-Wallis and Mann-Whitney *U* tests).

RESULTS

Citric Acid-Induced Cough

Compared with vehicle, 10 mg/kg memantine markedly reduced the number of cumulative coughs evoked by 0.01 to 0.3 mol/L citric acid ($P = .012$) (Fig 1A, Table 1). At 1 and 3 mg/kg, there was no significant effect of memantine over vehicle. No side effects associated with the 10 mg/kg memantine treatment were apparent. As expected, tripling the dose of memantine to 30 mg/kg similarly reduced cough responses but produced signs of neurologic effects and slight sedation.

In contrast to 10 or 30 mg/kg memantine, dextromethorphan (30 mg/kg; $n = 8$) and ketamine (30 mg/kg; $n = 8$) failed to produce a statistically significant reduction in cumulative coughs evoked by 0.01 to 0.3 mol/L citric acid ($P = .328$ and $P = .645$, respectively) (Fig 1B, Table 1). Dextromethorphan but not ketamine significantly reduced the cumulative number of coughs evoked by lower concentrations of citric acid (0.01-0.1 mol/L) ($P = .038$). Mild to moderate sedation was observed in 62.5% of the dextromethorphan-treated animals and 87.5% of the ketamine-treated animals. When administered at 30 mg/kg, the sedation produced by dextromethorphan and memantine was long lasting (> 2 h) and persisted throughout the citric acid challenge, whereas ketamine-induced sedation rapidly reversed during the cough challenge (40-50 min postinjection).

The 50 mg/kg doses of ketamine and dextromethorphan caused severe sedation, precluding assessment of cough responsiveness even though mean breathing frequency declined only slightly and insignificantly (control animals, 117.7 ± 26.3 breaths/min; ketamine, 92.3 ± 18.8 breaths/min [$P = .25$]; dextromethorphan, 90.0 ± 13.7 breaths/min [$P = .18$]). No significant change in tidal volume ($P = .293$) or expiratory time ($P = .14$) was found.

Baclofen (3 mg/kg) significantly reduced the cumulative number of coughs evoked by 0.01 to 0.1 mol/L

and 0.01 to 0.3 mol/L concentrations of citric acid ($P = .035$ and $P = .034$ vs control animals, respectively), whereas baclofen 0.3 mg/kg had no effect (Fig 2). Animals treated with 10 mg/kg baclofen ($n = 3$) were overtly sedated with difficulty standing, making cough challenge impossible. Respiratory rates were markedly reduced by 10 mg/kg baclofen (control animals, 120.5 ± 18.1 breaths/min; baclofen, 54.7 ± 20.6 breaths/min; $P = .014$), whereas expiratory time and tidal volume were unaffected ($P = .53$ and $P = .23$, respectively). Subjective signs of sedation were also apparent with 3 mg/kg baclofen treatments. Memantine 10 mg/kg and baclofen 3 mg/kg produced a similar reduction in the number of cumulative coughs ($P = .35$).

Relative to vehicle-treated animals, ondansetron (10 mg/kg intraperitoneal, $n = 4$) had no significant impact on citric acid (0.01-0.3 mol/L)-evoked coughing ($P = .39$) (e-Fig 1). This dose of ondansetron abolished serotonin-evoked cardiopulmonary responses (data not shown).

Bradykinin-Induced Cough

Compared with vehicle, memantine (10 mg/kg) substantially reduced the cumulative number of coughs evoked by 0.1 to 3 mg/mL bradykinin (0.0 [IQR, 0-0.8] vs 16 [IQR, 9.5-18.5] coughs for memantine and control animals, respectively, $P = .002$) (Fig 3). A single vehicle-treated animal coughed 14 times during pretreatment with the peptidase inhibitors but then had a similar response to other control animals during bradykinin challenge, coughing a further 15 times during inhalation of 3 mg/mL bradykinin.

Respiratory Parameters

With the exception of baclofen 10 mg/kg, none of the treatments studied altered breathing frequency or expiratory times (e-Fig 2) during citric acid challenge. Tidal volume was significantly increased with increasing citric acid concentration in all treatment groups. Bradykinin induced a small reduction in breathing frequency in control animals but a small increase in breathing frequency following 10 mg/kg memantine treatment ($P = .012$).

Responses to IV 2M5HT and Mecamylamine

Control animals showed no significant changes in mean arterial pressure, respiratory rate, or heart rate to boluses of 2M5HT at 10 μ g/kg, whereas 100 μ g/kg induced a fall in mean arterial pressure, a rise in respiratory rate, and still no change in heart rate. In memantine-treated animals (10 mg/kg), responses were similar except that the rise in respiratory rate with 2M5HT 100 μ g/kg was blunted and slower in

FIGURE 1. A, Cumulative number of coughs in response to citric acid aerosols in control animals compared with increasing doses of MEM. Compared with control animals, MEM 10 mg/kg significantly reduced the cumulative number of coughs ($P = .012$). B, Compared with control animals, 30 mg/kg KET and 30 mg/kg DEX did not significantly reduce the number of coughs evoked by 0.01 to 0.3 M citric acid ($P = .65$ and $P = .33$, respectively), but MEM 30 mg/kg did ($P = .013$). DEX and MEM but not KET significantly reduced the coughs evoked by lower concentrations of citric acid (0.01–0.1 M) ($P = .038$ and $P = .031$). DEX = dextromethorphan; IQR = interquartile range; KET = ketamine; MEM = memantine. $*P \leq .05$.

onset (e-Figs 3, 4). There were no significant differences whatsoever between control and memantine-treated animals in their responses to mecamlamine at doses of 1 or 3 mg/kg (e-Fig 5).

NMDA Subunit Expression

NR1 subunit gene expression was detected in all tissues evaluated (data not shown). PCR of neural tissue suggested some differences in expression of NR2 receptor subunit genes (Fig 4, Table 2, e-Table 1). Nodose ganglia and nTS rarely expressed the NR2A gene compared with 50% of jugular ganglia and most cerebellar samples. The NR2B subunit was, however,

present in most ganglia tissue and in 50% of nTS samples, whereas NR2C and NR2D expression was detected in the majority of these tissues. Lung tissue did not express NR2A, but other subunits were present.

DISCUSSION

To our knowledge, this study provides the first evidence that memantine has antitussive activity with significant inhibition of both citric acid- and bradykinin-induced cough in guinea pigs. The degree of inhibition was similar to that seen with baclofen

Table 1—Cumulative Number of Coughs With Increasing Concentrations of Citric Acid for Treatment Groups and Contemporaneous Control Subjects

Dose, mg/kg	Citric Acid Concentration		
	0.01 mol/L	0.1 mol/L	0.3 mol/L
Control subjects (n = 20)	0.0 (0.0-1.0)	3.0 (0.5-23.0)	24.0 (13.0-25.5)
Memantine (n = 5)	0.0 (0.0-1.0)	5.0 (1.0-20.0)	14.0 ^a
Memantine (n = 8)	0.0 (0.0-0.0)	3.0 (1.0-7.0)	24.5 (18.3-27.3)
Memantine (n = 6)	0.0 (0.0-0.0)	0.0 (0.0-1.8)	1.5 ^b (0.3-10.3)
Control subjects (n = 8)	0.0 (0.0-0.0)	16.0 (3.5-26.0)	19.5 (7.5-26.0)
Dextromethorphan (n = 8)	0.0 (0.0-0.0)	0.5 ^b (0.0-1.5)	7.5 (2.5-23.5)
Ketamine (n = 8)	0.0 (0.0-0.0)	1.5 (0.0-6.3)	11.0 (11.0-21.0)
Memantine (n = 6)	0.0 (0.0-0.0)	0.0 ^b (0.0-0.0)	1.5 ^b (0.0-5.5)
Control subjects (n = 7)	0.0 (0.0-1.0)	14.0 (1.0-23.0)	24.0 (13.5-25.0)
Baclofen (n = 8)	0.0 (0.0-0.0)	9.0 (0.8-15.8)	12.5 (1.8-23.8)
Baclofen (n = 8)	0.0 (0.0-0.0)	0.0 ^b (0.0-0.3)	5.0 ^b (4.0-13.0)
Control subjects (n = 12)	0.0 (0.0-0.0)	1.0 (0.0-12.5)	11.0 (4.5-22.25)
Ondansetron (n = 4)	0.0 (0.0-0.0)	6.5 (3.8-12.0)	21.5 (12.0-10.3)

Data are presented as median (interquartile range).

^aThere is no interquartile range for this value as only one animal inhaled the 0.3 mol/L concentration.

^bIndicates significant difference in cough from control animals.

FIGURE 2. Cumulative number of coughs in response to citric acid aerosols in control animals and with increasing doses of BAC. BAC 3 mg/kg significantly reduced the cumulative number of coughs evoked by 0.01 to 0.1 M and 0.01 to 0.3 M concentrations of citric acid ($P = .035$ and $P = .034$, respectively), whereas 0.3 mg/kg BAC had no demonstrable effect. BAC = baclofen. See Figure 1 legend for expansion of other abbreviation. * $P \leq .05$.

but without the associated sedation and was comparably more effective than high doses of other NMDAR antagonists. Because there was little evidence of significant activity at nicotinic acetylcholine or serotonin receptors, it is most likely that the inhibition of cough by memantine was NMDAR dependent. Receptor subunit expression suggested the presence of NMDARs in both the central and the peripheral tissues involved in the cough reflex, with some degree of differential expression of NR2 subunits that might permit targeting specific pathways in cough with NMDAR-selective blockade.

Coughing is known to be initiated by activation of vagal afferent fibers in the larynx and large airways through acid and mechanically sensitive A δ fibers and by activation of capsaicin-sensitive C fibers that are also responsive to acid (through the transient receptor potential vanilloid type-1 channels) and bradykinin.¹⁹ These fibers terminate in the nTS, where microinjection of NMDAR antagonists have been shown to significantly inhibit coughing in response to citric acid.^{20,21} The effect of memantine on both bradykinin- and citric acid-evoked cough is consistent with inhibition of the C-fiber pathway, but an additional effect on A δ fibers cannot be excluded.

NMDAR subunit gene expression was detected not only in the nTS, the primary site of vagal afferent termination in the brainstem, but also in vagal ganglia. The latter supports the notion that NMDARs may be present in the airway nerve terminals and may modulate neurotransmitter release presynapti-

cally in the CNS. Consistent with this hypothesis, we have observed NMDAR-dependent Ca^{2+} influx in dissociated nodose and jugular ganglia neurons projecting to the airways (data not shown). The differential expression of NR2A subunits in the jugular and nodose ganglia raises the possibility of targeting specific populations of afferent nerves therapeutically if these receptors can be shown to be functionally important in modulating cough. This assertion awaits a more systematic evaluation of NMDAR subunit expression in identified neurons.

Although NMDARs are reported to be mechanically sensitive²² and our PCR studies are consistent with a peripheral expression of NMDARs by vagal afferents, a central site of action for memantine seems most likely, especially as the antitussive effects of dextromethorphan are absent when delivered to the airway.^{7,23} Nevertheless, memantine treatment did not obviously cause sedation or suppress respiration at doses that almost completely inhibited coughing, which may be a consequence of the use-dependent action of memantine and NMDAR specificity at the doses used in this study. The selective effects of memantine on cough also might be explained by its greater affinity for NMDAR subtypes²⁴ or effects at extrasynaptic NMDARs.²⁵ In contrast, high doses of dextromethorphan and ketamine did not suppress coughing as effectively as memantine, despite clear evidence of sedation. Perhaps the relative lack of side effects seen with memantine treatment in this study is attributable to its inability to interact with the additional targets for dextromethorphan and ketamine

FIGURE 3. Cumulative number of coughs in response to bradykinin aerosols in control and MEM-treated animals. MEM 10 mg/kg significantly reduced coughing compared with control subjects ($P = .002$). See Figure 1 legend for expansion of abbreviations. * $P \leq .05$.

FIGURE 4. Representative polymerase chain reaction gel showing the presence of NR2 subunit genes A through D in nodose and jugular ganglia and nTS from a single animal. nTS = nucleus tractus solitarius.

(eg, sigma-1 receptors, hyperpolarization-activated cyclic nucleotide-gated 1 channels).^{2,26,27}

Baclofen was chosen as an additional positive control because it is known to suppress coughing in animals,²⁸ reduces cough reflex sensitivity in humans,²⁹ and has been used clinically to treat cough, although its utility is hampered by its sedative effects.³⁰ Indeed, animals treated with high-dose baclofen were overtly sedated, with depressed breathing frequency. Moderate doses of baclofen were better tolerated and produced antitussive effects similar to those of memantine without respiratory depression.

Other properties of NMDARs provide circumstantial evidence for a role in modulating the cough reflex. The female sex has a more sensitive cough reflex than the male sex when challenged with inhaled irritants³¹ and has higher frequency of coughing when experiencing chronic cough.³² Estrogens have significant influence on NMDARs. In the hippocampus, elevated estradiol levels are associated with increased synaptic receptor density, increased transmission, and enhanced long-term potentiation.^{33,34} Furthermore, cross-organ sensitization between the uterus and urethra is mediated by phosphorylation of NR2B subunits and modulated significantly by the estrous cycle.^{35,36} Such NMDAR-dependent interactions between organs may be analogous to the coughing associated with extrapulmonary disorders, such as gastroesophageal reflux disease.³⁷

In conclusion, this study provides initial evidence that memantine has antitussive activity that is most likely NMDAR dependent, a mechanism previously shown to translate from animal models to clinical effects on cough in humans. Memantine, therefore,

Table 2—Proportion of Tissues, Taken From Three to Eight Animals, Expressing the Genes for the NR2 Subunits A Through D

	Nodose	Jugular	nTS	Trachea	Parenchyma	Cerebellum
GRIN2A	1/8	4/8	1/4	0/3	0/4	5/6
GRIN2B	6/8	8/8	2/4	1/3	2/4	5/6
GRIN2C	6/8	7/8	3/4	2/3	4/4	5/6
GRIN2D	5/7	5/7	5/5	2/3	1/3	5/6

GRIN2 = glutamate receptor, ionotropic, N-methyl D-aspartate 2 (A-D); nTS = nucleus tractus solitarius.

has the potential to be a safe, effective, and well-tolerated treatment not only for patients with acute cough but also, perhaps, for patients with chronic intractable coughing where there are few effective treatment options.

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Author contributions: Dr Canning had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Dr Smith: contributed to the study design; acquisition, analysis, and interpretation of data; drafting and revisions of the manuscript; and approval of the final version of the manuscript.

Dr Hilton: contributed to the study design; acquisition, analysis, and interpretation of data; revisions of the manuscript; and approval of the final version of the manuscript.

Ms Saulsberry: contributed to the study design; acquisition, analysis, and interpretation of data; revisions of the manuscript; and approval of the final version of the manuscript.

Dr Canning: contributed to the original concept of the study; study design; acquisition, analysis, and interpretation of data; revisions of the manuscript; and approval of the final version of the manuscript.

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