Effects of Central Muscarinic-1 Receptor Stimulation on Blood Pressure Regulation

Aharon Medina, Neil Bodick, Ary L. Goldberger, Margaret Mac Mahon, Lewis A. Lipsitz

Abstract Stimulation of central nervous system muscarinic-1 (M1) receptors in animals increases blood pressure, heart rate, and sympathetic outflow. In Alzheimer’s disease, stimulation of central M1 receptors is reduced. When the oral formulation of the selective M1 agonist xanomeline was tested for the treatment of Alzheimer’s disease, an increased incidence of syncope was observed. Therefore, we used Alzheimer’s disease as a model of relative M1 deficiency to determine the effect of M1 receptor stimulation on blood pressure regulation in humans. Eight Alzheimer’s patients and 6 healthy age- and sex-matched subjects underwent blood pressure, heart rate, forearm vascular resistance, plasma norepinephrine, and heart rate variability measurements during 90 minutes after ingestion of xanomeline or placebo, then during 45 minutes of head-up tilt. Alzheimer’s patients were studied on three occasions: after placebo, the first dose of xanomeline, and 3 days of xanomeline. Normal subjects were studied after placebo and the first dose of xanomeline. A subset of 5 Alzheimer’s patients was studied with the peripheral muscarinic antagonist methscopolamine. Oral xanomeline increased supine systolic and diastolic blood pressures in normal subjects and heart rate and plasma norepinephrine in all subjects. During the placebo tilt, 0 of 8 Alzheimer’s patients and 2 of 6 healthy subjects developed near-syncope, and during the first-dose xanomeline tilt, 4 of 8 Alzheimer’s patients and 3 of 6 healthy subjects had near-syncope. The maximal decrease in systolic blood pressure during tilt was greater with xanomeline than placebo in both groups (P<.03). Methscopolamine did not prevent xanomeline-induced hypotension. Central M1 receptor stimulation with the oral formulation of xanomeline in humans is associated with symptomatic stimulation under supine conditions and impaired baroreflex compensation during tilt. Alzheimer’s patients, who presumably lack M1 receptor activity, may have a reduced risk of tilt-induced syncope compared with normal subjects. Both groups, however, have enhanced susceptibility to hypotension and syncope when M1 receptor activity is pharmacologically increased. (Hypertension. 1997;29:828-834.)

Key Words • orthostatic hypotension • syncope • tilt • xanomeline • aging • neurotransmitters • Alzheimer’s disease

Over the past decade, the role of muscarinic receptors in health and disease and the potential therapeutic value of various cholinergic agonists and antagonists have received increasing attention.1,2 For example, tacrine hydrochloride, a centrally active acetylcholinesterase inhibitor that prevents the degradation of acetylcholine in the brain, has recently been approved for the treatment of Alzheimer’s disease.3 Currently, drugs that are selective for M1 receptors in the brain are undergoing clinical trials for the treatment of this disease. A recent phase 2 trial of the selective, centrally active M1 agonist xanomeline tartrate4 resulted in an unexpectedly high incidence of syncope among more than 300 patients with Alzheimer’s disease exposed to the oral formulation of the drug.5 Although tacrine has not been reported to affect cardiovascular function, it is possible that the selective M1 agonist xanomeline may do so.

Animal studies suggest that central muscarinic receptor stimulation plays an important role in cardiovascular regulation.7,8 Intracerebral administration of muscarinic agonists into the posterior hypothalamic nucleus9 or medulla10 in rats has been shown to evoke pressor9,10 and cardioacceleratory11 responses that are inhibited by the selective M1 agonist pirenzepine. Circulating catecholamines and sympathetic neural outflow10 are elevated in animals after central muscarinic stimulation. Furthermore, central M1 receptor activation appears to have an important role in mediation of the baroreflex12 and the Bezold-Jarisch reflex13 in rats. Thus, alterations in M1 receptor activity in the brain may influence autonomic nervous system control of cardiovascular function.

The M1 muscarinic receptor is one of five known muscarinic subtypes in the cholinergic nervous system.14,15 In Alzheimer’s disease, this receptor is preserved in the cerebral cortex and hippocampus despite the loss of presynaptic cholinergic neurons projecting from the nucleus basalis of Meynert.16,17 If cholinergic receptors mediate sympathetic outflow from the central nervous system, the loss of M1 receptor stimulation in Alzheimer’s disease would be expected to result in BP alterations. In rats, depletion of brain acetylcholine with hemicholinium-3 resulted in a decrease in BP.8 Patients with Alzheimer’s disease have lower BPs than age- and sex-matched controls,18 and BP falls as the disease progresses.19 Therefore, Alzheimer’s disease provides a possible model for studying the cardiovascular effects of deficiency and restoration of M1 receptor activity in the brain.

The purpose of this study was to determine the cardiovascular effects of central M1 receptor stimulation during supine rest and orthostatic stress. Accordingly, cardiovascular measurements were obtained in the supine and head-up tilt positions during oral xanomeline and placebo treatments in groups of Alzheimer’s patients and age-matched healthy subjects. To exclude any potential

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From the Hebrew Rehabilitation Center for Aged Research and Training Institute, the Beth Israel/Deaconess Medical Center Department of Medicine, Harvard Medical School, Boston, Mass, and Eli Lilly Laboratory for Clinical Research, Indianapolis, Ind.

Reprint requests to Lewis A. Lipsitz, MD, Hebrew Rehabilitation Center for Aged, 1200 Centre St, Boston, MA 02131. E-mail Lipsitz@Mail.HHCA.Harvard.edu.

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Peripheral muscarinic effects of the drug, a subset of Alzheimer’s patients was studied with the combination of xanomeline and the peripherally active nonselective muscarinic antagonist methscopolamine, which does not cross the blood-brain barrier.21

Methods

Two studies were conducted. The main study examined the effects of xanomeline on cardiovascular function, and a substudy examined the effect of methscopolamine on xanomeline-related syncope.

Subjects

In the main study, 8 patients with NINCDS-ADRDA (National Institute of Neurological and Communication Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association)-defined Alzheimer’s disease22 and 6 age- and sex-matched healthy subjects with normal cognitive function were studied in the Beth Israel Hospital CRC. Alzheimer’s patients were recruited from among those who participated in a previous Eli Lilly Co-sponsored clinical trial of xanomeline. They were enrolled in the study after they had been off xanomeline for at least 1 week (half-life is 4 to 5 hours). Four of these patients had experienced syncope while taking the drug. Their clinical evaluations failed to reveal another cause of syncope.

For the substudy of muscarinic blockade, 5 Alzheimer’s patients were admitted to the CRC for studies with xanomeline and methscopolamine, alone or in combination. Four of these subjects had hypertension and syncope during the previous tilt study on xanomeline, and 1 experienced syncope while taking the drug during the clinical trial. Healthy volunteers were recruited from the Harvard Cooperative Program on Aging registry. All subjects, including Alzheimer’s patients and healthy control subjects, were carefully screened for the absence of cardiovascular diseases, arrhythmias, or medications potentially associated with syncope. All the subjects, or their legal guardians in cases of Alzheimer’s disease, signed an informed consent. The study was approved by the Institutional Review Board of Beth Israel Hospital, Boston, Mass.

Protocol

For the main study, patients with Alzheimer’s disease were admitted to the CRC for 7 days, during which they underwent three tilt studies. The first tilt was conducted on day 2, 90 minutes after they had received oral placebo capsules; the second on day 3, 90 minutes after the first dose of oral xanomeline; and the third on day 6, after 3 days of xanomeline treatment. Since one of the purposes of the study was to assess the first-dose and chronic dosing effects of xanomeline, the order of these tests could not be randomized without an unacceptably long stay in the CRC.

The xanomeline dose was the highest dose tolerated without gastrointestinal side effects during the previous clinical trial. Five patients received 75 mg, 2 patients 50 mg, and 1 patient 25 mg, each three times daily. All subjects with Alzheimer’s disease underwent continuous ambulatory cardiac monitoring during the entire 7-day stay in the CRC.

Healthy volunteers were admitted to the CRC for 3 days, during which they underwent the identical tilt protocol with placebo on day 2 and 75 mg xanomeline on day 3. Since the first-dose and 3-day xanomeline effects were similar in Alzheimer’s patients, a longer stay in the CRC could not be justified. Continuous cardiac monitoring was performed for 5 to 6 hours during each tilt study.

For the substudy of peripheral muscarinic blockade, the 5 Alzheimer’s patients were admitted to the CRC for 6 days. They underwent three identical tilt studies; one with xanomeline alone, one with methscopolamine alone, and one with a combination of the two. The drugs were prepared by the hospital pharmacy and administered in a double-blind randomized fashion on days 2, 4, and 6. The xanomeline dose was 75 mg on the morning of the study. The methscopolamine dose was 5 mg at 8 pm and 8 am before each study. The 5-mg dose of methscopolamine was twice the usual oral dose for gastrointestinal motility disorders.21 It was given on two occasions to ensure adequate absorption. An identical-appearing placebo was given instead of methscopolamine or xanomeline when only a single drug was being tested.

Data Acquisition and Tilt Study

On the morning of each study, an ambulatory cardiac monitor (Marquette Electronics) was connected to the subject. Baseline data were collected during 30 minutes in the supine position. Then the subjects sat up for a standardized 1760-KJ liquid meal (74% carbohydrate, 6% fat, 20% protein).23 Drug or placebo was given 10 minutes after the start of the meal to minimize the gastrointestinal side effects of the xanomeline. The tilt study was conducted 90 minutes later at the time of peak drug effect. Subjects were tilted head-up to 60° for 45 minutes or until the onset of symptoms.

Data acquisition included continuous HR recording, measurements of BP every 5 minutes supine and every 3 minutes during tilt, forearm vascular resistance (FVR) measurements at 15-minute intervals by venous occlusion plethysmography,24 and measurements of plasma norepinephrine levels (two at baseline, 20 minutes after the drug/placebo, and 10 minutes before and 20 minutes after the tilt). Since many of the subjects developed syncope requiring termination of the tilt before the 20-minute norepinephrine sample, we could not evaluate the norepinephrine response to tilt. Continuous ECG recordings were examined for evidence of arrhythmias during the procedure and were also used for the calculation of high- and low-frequency HR spectral power to assess autonomic control of HR.

Spectral Analysis of HR Variability

Continuous ECG data were digitized at 125 Hz. Ten-minute sections of stationary continuous ECG recordings were selected for analysis at the following time points: before the study; immediately before the tilt; during the tilt; and for Alzheimer’s patients, during the midafternoon and at midnight.

Each heartbeat was annotated by an automated arrhythmia detection algorithm25 and verified by visual inspection. Occasional ectopic beats were deleted, and normal beats were substituted by linear interpolation. HR was calculated as the reciprocal of the RR interval. Instantaneous, uniformly sampled HRs were obtained by resampling each time series at 2 Hz. Then, each time series was analyzed with a fast Fourier transform algorithm yielding a 512-point power spectrum. Total spectral power, an index of overall HR variability, was computed (in arbitrary units) for the entire 0.01- to 0.50-Hz frequency spectrum. Low-frequency power, a measure of both sympathetic and parasympathetic control of HR, was calculated for the 0.01- to 0.15-Hz frequency band. High-frequency power, representing parasympathetic regulation of HR, was determined for the 0.15- to 0.50-Hz band at each time segment.

Data Analysis

For all cardiovascular variables and spectral indices, baseline values and their changes in response to drug and head-up tilt were compared between treatments and between Alzheimer’s patients and healthy subjects by two-factor (treatment or group and time) repeated-measures ANOVA. Maximal change in BP during tilt and standing was compared between the groups by the Wilcoxon signed-rank test. Statistical significance was set at a level of $p<.05$. All data are expressed as mean±SEM.
Baseline Subject Characteristics

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Values are mean±SEM.

Results

Main Study: Baseline Subject Characteristics

Subject characteristics are summarized in the Table. There were no significant differences between Alzheimer’s patients and healthy volunteers in age, sex distribution, height, or resting supine HR, BP, FVR, or plasma norepinephrine levels. Alzheimer’s patients tended to have a higher weight than healthy volunteers (P=0.09). All healthy volunteers had a Mini-Mental Status Examination score of 30/30.

Effects of Xanomeline During 90 Minutes Supine

Healthy subjects. The hemodynamic and plasma norepinephrine responses to xanomeline for healthy subjects are shown in Fig 1. SBP (P=0.02), DBP (P=0.004), MABP (P=0.006), and HR (P=0.001) increased by 90 minutes after the administration of xanomeline but not after placebo. FVR was similar after xanomeline and placebo. Mean plasma norepinephrine levels were similar at baseline and tended to increase more during the 90 minutes after xanomeline (863±29 pmol/L [146±37 pg/mL]) than with placebo (425±106 pmol/L [72±18 pg/mL], P=0.09 versus xanomeline).

Alzheimer's patients. The hemodynamic and plasma norepinephrine responses to xanomeline for Alzheimer's patients are shown in Fig 2. There was a statistically significant increase in plasma norepinephrine after the first dose of xanomeline (P=0.04) and in both HR and norepinephrine after 3 days of treatment (P=0.02). Xanomeline had no significant effect on BP or FVR in Alzheimer’s patients.

Comparison between Alzheimer's patients and healthy subjects. There were no significant differences in SBP, DBP, MABP, HR, FVR, or plasma norepinephrine levels between Alzheimer’s patients and healthy subjects over 90 minutes after administration of either placebo or xanomeline.

Effects of Xanomeline During Upright Tilt

Healthy subjects. Two of the 6 healthy subjects experienced a presyncopal event during tilt on placebo after 9 and 16 minutes in the upright position. These subjects had a similar episode during tilt on xanomeline, but it occurred sooner, after 3 and 12 minutes, respectively. A third subject had a presyncopal episode only on xanomeline treatment. Before the event, the subjects became restless, pale, clammy, and diaphoretic. SBP was 60 to 70 mm Hg, and HR fell by 6 to 19 bpm during each episode. All subjects recovered spontaneously in the supine position.

The maximal decrease in SBP during tilt was 28±7 mm Hg on placebo versus 52±10 mm Hg on xanomeline (P=0.03) (Fig 3). Orthostatic hypotension was still present.

Fig 1. Normal subjects: SBP, HR, plasma norepinephrine levels, and FVR during treatment with placebo (♦) and xanomeline (▲). Data obtained during supine rest and initial phase of tilt are shown for all variables except norepinephrine (supine only). Note parallel, gradual increases in SBP, HR, and norepinephrine with xanomeline (P=0.02, P=0.001, and P=0.09, respectively).
5 hours after the xanomelone dose; SBP fell an average of 24±5 mm Hg after 3 minutes of standing while taking xanomelone versus 1±5 mm Hg on placebo (P=0.03). HR and FVR increased significantly during tilt (P=0.001) and P=0.01, respectively) (Fig 1). HR was significantly higher during xanomelone treatment than placebo (P=0.01); however, the relative change in HR during tilt was similar. No treatment effect was noted for FVR (Fig 1).

Alzheimer's patients. In the placebo study, all 8 Alzheimer's patients tolerated 45 minutes of upright tilt without syncope or hypotension. However, after the first dose of xanomelone, 4 patients developed presyncope with clinical symptoms similar to those of the healthy subjects. In each event, SBP fell to <75 mm Hg and HR fell by 2 to 11 bpm. Three of these subjects had the same presyncope and hypotensive response after 3 days of xanomelone treatment. The maximal decline in SBP during tilt after the first dose was 50±19 mm Hg on xanomelone versus 15±6 mm Hg on placebo (P=0.04) (Fig 3). Mild orthostatic hypotension was still present 8 hours after the xanomelone dose; SBP fell an average of 7±4 mm Hg after 3 minutes of standing while on xanomelone versus 0.5±4 mm Hg on placebo (P=0.05). Similar results were obtained after 3 days of xanomelone treatment.

HR and FVR increased significantly during all tilt studies (P=0.001 and P=0.03, respectively) (Fig 2). Unfortunately, the vascular response could not be reliably determined at the onset of symptoms because of the need to return subjects immediately to the supine position. There was no difference in the HR or FVR response to tilt during xanomelone and placebo treatments.

Comparison between Alzheimer's patients and healthy subjects. During the placebo tilt study, 2 of the 6 healthy subjects developed syncope and hypotension, in contrast to none of the 8 Alzheimer's patients (Fig 4). Furthermore, during the first 6 minutes of the placebo tilt, while all the subjects were still upright, SBP declined to a greater extent in normal subjects than in Alzheimer's patients (P=0.02). No significant differences were noted in HR, FVR, or plasma norepinephrine.

During xanomelone administration, 50% of both Alzheimer's patients and normal subjects experienced presyncope and hypotension (Fig 4). There were no significant differences in any of their hemodynamic parameters.

HR Variability

Alzheimer's patients. Spectral analysis of HR variability revealed a significant decrease in total, low-frequency,
and high-frequency power during xanomeline treatment (Fig 5). This effect started in the afternoon after the acute dose ($P=0.04$ and $P=0.02$ for low- and high-frequency power, respectively) and was present during the night ($P=0.09$ and $P=0.001$, respectively). Standard deviation of the HR also decreased in the afternoon after xanomeline ($P=0.05$).

**Healthy subjects.** High-frequency HR variability tended to be lower during the tilt study with xanomeline treatment, but this did not reach statistical significance ($P=0.12$).

### Ambulatory Cardiac Monitoring

A significant increase in HR occurred during xanomeline treatment in all subjects ($P<0.0001$). This effect was present after the first dose of xanomeline on day 3, and it persisted until the end of the study (day 6 for Alzheimer’s patients and day 3 for control subjects). There was no evidence of important cardiac arrhythmias or conduction abnormalities, including at the time of presyncope. No significant ST-segment changes were noted in any of the subjects.

### Effect of Peripheral Muscarinic Blockade

Methscopolamine produced a significant increase in HR during supine rest before tilt ($P=0.03$). When xanomeline alone was administered, the maximal decrease in SBP during tilt was similar to what it was in the main study, 45±6 mm Hg. With methscopolamine alone, SBP declined by 33±5 mm Hg during tilt ($P=0.04$ versus xanomeline alone). When the patients were tilted with a combination of xanomeline and methscopolamine, the decline in SBP was 43±9 mm Hg, no different from xanomeline alone ($P=0.04$ versus methscopolamine alone).

Three patients had near-syncpe during tilt, 1 on xanomeline alone and 2 others with the combination. Thus, peripheral muscarinic blockade with methscopolamine did not prevent tilt-induced hypotension associated with xanomeline.

### Discussion

The main results of this study may be summarized as follows.

1. In healthy volunteers at rest, central M₁ receptor stimulation with the oral formulation of xanomeline is associated with increases in BP, HR, and plasma norepinephrine levels, suggesting sympathetic stimulation. Car-
spectral power in Alzheimer's patients. These findings are consistent with the known acute effects of central nervous system M1 receptor stimulation in animals, which results in increased sympathetic outflow and elevated BP, HR, and circulating catecholamines. They are also supported by one previous study in psychiatric patients showing that physostigmine, which increases central nervous system acetylcholine levels, also increases HR and BP.

Different mechanisms have been proposed to explain the effect of central M1 receptor stimulation on sympathetic outflow. Animal studies suggest that this effect may be mediated by stimulation of α1-receptors in the nucleus tractus solitarii of the hypothalamus. In rats, the α1-blocker prazosin inhibited thepressor response obtained after intracerebral administration of the cholinesterase inhibitor neostigmine. Another animal study suggested that the M1 pressor response may be mediated through serotonin receptors in the posterior hypothalamus.

Information about central muscarinic receptor function in humans is very limited. Intracerebral arecoline administration for the treatment of Huntington's disease in 6 patients pretreated with methscopolamine caused a significant dose-related increase in SBP and HR. Pirenzepine, a selective M1 antagonist, caused bradycardia in healthy volunteers. This was reversed by high doses of atropine. To the best of our knowledge, the present study is the first to report the cardiovascular effects of direct pharmacological stimulation of central M1 receptors in humans.

Although the administration of xanomeline was shown to increase indirect measures of sympathetic activity, such as SBP, HR, and plasma norepinephrine, the presumed deficiency of central M1 activity in Alzheimer's patients did not appear to affect baseline values of these measures (Table). These indirect measures may not be sensitive to small differences in the effects of endogenous M1 activity during basal conditions, or it is possible that differences in M1 receptor density are evident only during stimulated conditions such as head-up tilt.

Despite an apparent increase in sympathetic nervous system activity during supine rest, our results indicate that oral xanomeline provokes orthostatic hypotension. One possible mechanism for this paradoxical effect is M1 receptor-mediated provocation of the Bezold-Jarisch reflex. This reflex is evoked by stimulation of mechanoreceptors or chemoreceptors in the posterior wall of the left ventricle, resulting in vagally mediated hypotension and bradycardia. During upright tilt, vigorous ventricular contraction around a relatively empty chamber is believed to activate this reflex. In rats, central M1 receptor activity appears to be necessary to elicit the Bezold-Jarisch reflex; when M1 receptors are blocked by pirenzepine, the bradycardic response is abolished.

**Study Limitations**

A potential limitation of this study is its relatively small number of patients. Nevertheless, the BP, HR, and symptomatic responses to xanomeline were sufficiently large to achieve statistical significance. Because of the expense and difficulty of recruiting and intensively studying Alzheimer's patients, we could not justify a larger sample size.

Another potential limitation is the possibility of cross-activation of other muscarinic receptors by xanomeline. However, the drug has been shown in vitro and in vivo to be highly selective for M1 receptors in therapeutic doses.

Furthermore, this study included concomitant administration of xanomeline and the nonselective peripheral muscarinic blocker methscopolamine to exclude potential peripheral muscarinic effects.

Although our subjects were otherwise well matched, Alzheimer's patients tended to be heavier than the normal subjects. To the best of our knowledge, there is no reason to believe that this difference in body habitus might have rendered Alzheimer's patients less susceptible to orthostatic hypotension.

Finally, it is possible that the subjects may not have returned to baseline autonomic function by 24 hours after they experienced vasodepressor reactions. We were unable to randomize the sequence of tilt tests because of the necessary drug washout period between studies and the practical difficulty of keeping Alzheimer's patients in the CRC for a longer stay. The similarity of supine cardiovascular variables before the first and second tilt tests for both Alzheimer's patients and normal volunteers suggests that all subjects had returned to baseline within 24 hours.

Our findings are preliminary, but they suggest that central M1 receptor stimulation in humans has a profound effect on BP regulation. Furthermore, our results may have important clinical implications; pharmacological blockade of M1 receptors in the brain may have value in treating orthostatic hypotension or preventing vasodepressor syncope.

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**References**


