Scopolamine Increases Vagal Tone and Vagal Reflexes in Patients After Myocardial Infarction

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Objectives. The goal of this study was to assess the hypothesis that transdermal scopolamine would increase vagal activity in patients after myocardial infarction.

Background. In postmyocardial infarction patients, low heart rate variability and reduced baroreceptor reflex sensitivity are associated with increased mortality. Accordingly, there is an increasing interest in a mechanism for shifting the sympathovagal balance toward vagal dominance.

Methods. The effects of transdermal administration of scopolamine on heart rate variability and baroreceptor reflex sensitivity were assessed in 20 patients (mean age 59 ± 11 years) by pharmacologic washout 14 ± 3 days after myocardial infarction. Heart rate variability and baroreceptor reflex sensitivity were measured 24 h after application of the scopolamine patch and compared with the values measured before scopolamine and after application of a placebo patch. The following variables were derived from a 15-min electrocardiographic recording: the mean RR interval and its standard deviation, the mean square successive difference, the percent of intervals differing >50 ms from the preceding interval and the low and high frequency areas resulting from power spectral analysis.

Results. The placebo patch had no effect on the variables measured. Scopolamine increased both heart rate variability and baroreceptor reflex sensitivity significantly. Specifically, the mean RR interval and its standard deviation increased by 7.1% (p = 0.01) and 25% (p = 0.004), respectively. The mean square successive difference increased by 38% (p = 0.0003) and the percent of intervals differing >50 ms from the preceding interval by 100% (p = 0.001). The ratio of low to high frequency areas of the power spectrum decreased by 24% (p = 0.02), and baroreceptor reflex sensitivity increased by 42% (p = 0.0006). These effects were also evident in patients with very low initial values. Side effects were minimal.

Conclusions. Transdermal scopolamine increased measures of heart rate variability and baroreceptor reflex sensitivity in patients with a recent myocardial infarction toward values associated with a better prognosis. Pharmacologic modulation of the autonomic balance by scopolamine or related drugs deserves evaluation as a new and promising approach to reduce risk after myocardial infarction.

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The potential beneficial effects of augmented vagal activity during acute myocardial ischemia have received increasing attention in the past several years (1-4). Both experimental (5-7) and clinical studies (8-10) indicate a strong correlation between indexes of either impaired vagal reflexes or reduced vagal tone and a greater incidence of sudden cardiac death. Experiments performed in a conscious canine animal model for sudden cardiac death (11) have indicated that a depressed baroreflex sensitivity (5,6) and a reduced heart rate variability (7) characterize subjects at high risk for the occurrence of ventricular fibrillation. The experimental findings are closely paralleled by clinical studies in patients after myocardial infarction (8-10). These considerations, together with the negative effect on survival caused by the administration of traditional antiarrhythmic drugs (12,13), have given impetus to the search for a mechanism to shift the autonomic balance toward vagal predominance as a potential strategy in the prevention of sudden cardiac death.

Low doses of atropine and other muscarinic antagonists have been shown to produce paradoxic vagomimetic effects (14,15). Dibner-Dunlap et al. (16) observed that transdermal administration of the muscarinic antagonist scopolamine in healthy young men produced an increase in the mean RR interval and its standard deviation (SD) and in the baroreceptor response to graded neck suction. However, the relevance of these data to patients after myocardial infarction is uncertain because patients with myocardial infarction are older and show a transient impairment in vagal activity (6,17,18). Therefore, the goal of this study was to evaluate the effects of transdermal scopolamine on indexes of vagal tone and reflexes in patients shortly after myocardial infarction. Preliminary data have been presented elsewhere (19).

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Methods

The study was performed in 20 randomly selected patients who gave informed consent to the study (one additional patient declined to take part in the study, and one did not perform the study because of high blood pressure on the day of the first test). Two subjects were women, and 18 were men (mean age 59 ± 11 years; range 37 to 78; mean left ventricular ejection fraction 52 ± 12%, range 29% to 68%). The infarction was anterior in 8 patients and inferior in 12.

Exclusion criteria were age ≥80 years; atrial fibrillation or frequent atrial or ventricular arrhythmia that made analysis of sinus cycles impossible; atrioventricular block; history of insulin-dependent diabetes mellitus and signs or history of central or peripheral neuropathy; blood pressure >160/100 mm Hg with diuretic agents; and serious, concomitant noncardiovascular disease, such as renal or hepatic failure. Patients were also excluded if they had postinfarction angina, were directed to urgent mechanical or surgical revascularization or had heart failure unresponsive to the sole administration of diuretic agents. Patients were enrolled 8 to 15 days after myocardial infarction and were studied during pharmacologic washout. Beta-adrenergic blocking agents; calcium channel antagonists; vasodilators; antiarrhythmic, antidepressant and antihypertensive agents; nitrates, and any drug that could have interfered with the autonomic assessment were withdrawn at least 5 half-lives before the beginning of the study. No patient was receiving digitalis. Diuretic agents were allowed, if needed, to control blood pressure and pulmonary congestion. Platelet-active and noncardiovascular drugs were also allowed and kept at the same dosage throughout the study.

The protocol consisted of the assessment of heart rate variability and baroreceptor reflex sensitivity in control conditions, 24 h after the application of one patch of scopolamine (Transcop, Recordati) to the skin behind one ear and 24 h after removal of the active compound patch and its replacement with a placebo patch. The patients had no knowledge of the treatment given. The active patch contained 1.5 mg of scopolamine and was designed for continuous release of 0.5 mg every 24 h. Although most of the tests in each patient were performed on 3 consecutive days, occasionally >1 day elapsed between two tests for logistic reasons. However, the patch was always applied 24 h before the evaluation. The test with scopolamine was performed 14 ± 3 days (range 10 to 18) after the occurrence of myocardial infarction.

The test was always performed at the same time of day, ≥3 h after a light meal. Throughout the study, smoking and caffeine-containing beverages were not allowed. The patients were studied while supine at rest in a quiet, temperature-controlled room, and they were asked to relax and to avoid talking and sleeping. The recording was started after 10 to 15 min of acclimation and lasted 15 min. Heart rate variability was assessed with a commercially available software (Predictor, Corazonix). Analog electrocardiographic (ECG) signal was digitized at 500 Hz. The QRS complex was recognized by cross correlation with the template of the normal QRS complex chosen by the investigator. Premature atrial and ventricular beats and the subsequent interval were excluded automatically by the analysis, and the automatic detection was also visually checked by the investigator for proper identification. Time domain variables considered in this study were the mean RR interval and its SD, the mean squared successive difference and the percent of intervals differing >50 ms from the preceding interval. Power spectral analysis was performed by an autoregressive algorithm (Predictor, Corazonix) with a direct current filter and model order 16. The boundaries of the low frequency (average 0.05 to 0.16 Hz, with a peak at 0.1 Hz) and high frequency (average 0.18 to 0.4 Hz, with a peak at 0.27 Hz) regions were chosen manually for each test by an investigator unaware of the treatment, and the area was automatically obtained by the software. A very low frequency peak was generally not revealed by the analysis. A pilot study of the reproducibility of the time domain measures (the SD of the mean RR interval, the mean square successive difference and the percent of intervals differing >50 ms from the preceding interval) among two recordings ≤90 min from each other, performed with this protocol in 10 patients, shows a correlation (r value) from 0.974 to 0.982.

Assessment of baroreceptor reflex sensitivity was performed shortly after, according to the method of Smyth et al. (20). Blood pressure was continuously monitored noninvasively by infrared digital plethysmography (Finapres 2300, Ohmeda). This signal and a lead II ECG signal were digitized and fed into a Compaq 286 computer with custom-made software (G. Pinna, PhD, Montescano, Italy) for analysis of baroreceptor reflexes. The alpha,-agonist phenylephrine was injected (initial dose 200 fag) for 30 s to obtain an increase in blood pressure of 20 to 30 mm Hg. Baroreceptor reflex sensitivity was calculated as the slope of the linear regression line relating systolic blood pressure changes to RR interval changes. Regression lines with ≥20 points and an r ≥ 0.70 were accepted for analysis. The phenylephrine injection was repeated until three linear regressions with these characteristics were obtained (in most cases this was achieved with the first three injections). The average of these three slopes was then taken as the baroreceptor reflex sensitivity value. The average duration of the whole test session was 80 min (range 70 to 100).

Statistical analysis. Results are expressed, unless otherwise stated, as mean value ± SD. Regression lines for baroreceptor reflex sensitivity were calculated with the least-squares method. Normality of the distributions was assessed both graphically and with a goodness of fit test. The overall difference among mean values in the three groups (control, scopolamine, placebo) was evaluated with one-way analysis of variance for repeated measures or with a Kruskal-Wallis test for nonnormal distributions (percent of intervals differing >50 ms from the preceding interval and power spectral densities). A subsequent direct comparison
was made, respectively, with a t test for paired samples or with a Wilcoxon signed rank test. A p value < 0.05 was considered significant, with the exception of the multiple comparisons among the three treatments, where the limit for significance was set at p < 0.017, according to the Bonferroni correction.

**Results**

The scopolamine patch was well tolerated by the patients. The sole side effects were dryness of mouth in one patient and light-headedness in another patient. The latter patient also reported a similar effect during the subsequent placebo patch application. No other side effect was reported.

The overall technical quality of the tests was good. No heart rate variability recording had to be discarded because of excessive premature beats. The average correlation coefficient of the baroreceptor slopes was 0.85 ± 0.08. Three patients had on, two acceptable regression slopes in one of the two conditions. No major side effect was observed after phenylephrine injection. Two patients reported mild transient flushing; one had modest horripilation; and a few perceived the slowing of heart rate.

**Heart rate variability.** Analysis of variance for time domain variables derived from the 15-min recording of RR intervals revealed significant differences in all of the variables (Fig. 1). The placebo patch did not induce any significant change in any of the variables.

Mean heart period (mean RR interval) during the scopolamine test was greater compared with both control (average 7.1%, p = 0.01) and placebo conditions (average 9.9%, p = 0.0001). This effect on the RR interval corresponds to an average decrease of 5 to 6 beats/min in heart rate. The SD of the mean RR interval increased correspondingly more. During the scopolamine test, it was 25% greater than during the control period (p = 0.004) and 36% greater than during the placebo period (p = 0.003). This greater increase in the SD of the mean RR interval, compared with that of the mean RR interval, indicates that the increase in variability was linked only to a minor extent to the increase in mean heart cycle. The mean squared successive difference among heart cycles increased significantly during the scopolamine test compared with both the control (by 38%, p = 0.0003) and placebo conditions (by 61%, p = 0.0003). The percent of intervals among sinus beats differing >50 ms from the previous interval was significantly greater in the scopolamine test than in the control or placebo trial (p = 0.001 and p < 0.001, respectively, by the Wilcoxon signed rank test). Figure 2 is an example of the heart period histogram of a patient during control conditions and after scopolamine.
Analysis of the frequency domain variables (power spectrum density of the different areas) disclosed no overall difference among the three groups in the total (0 to 0.5 Hz) spectral density or in the low frequency component. A trend toward a difference was noted for the high frequency component (p = 0.06). The ratio of low and high frequency components showed a significant difference among the three groups (p < 0.05). The placebo conditions had no effect on this variable, whereas scopolamine induced a decrease that was of borderline significance compared with the value during control conditions (24%, p = 0.02) and was clearly significant compared with that during placebo conditions (p = 0.009) (Fig. 3). There was a moderate correlation between measures of heart rate variability during control conditions and after the scopolamine test (for the SD of the mean RR interval, r = 0.59, p = 0.005).

Figure 2. Example of frequency (FREQ) histogram and related time domain measures provided by the study software in one patient during control conditions (upper panel) and scopolamine treatment (lower panel). In this patient there was a very modest effect on the mean RR interval (Mean) but a marked effect on the standard deviation (StD) (+63%), the mean squared successive difference (MSSD) (+74%) and the percent of sinus cycles differing from the preceding cycle by >50 ms (PNN50) (+170%). HR = heart rate. The software also provides the total number of intervals that occurred during the recording (Ints); the number of those accepted for the analysis (Accept); the duration of the shortest (Short) and longest (Long) interval; the frequency of occurrence of the most common class of beats (MaxFreq), according to the selected bin width (BinWd, in the example 16 ms); and the following statistical variables obtained from the RR intervals (RR STATS): the mode and median (Med) of the distribution; the coefficient of variance (CoVr) and the standard deviation of the 5-min average normal to normal (NN) intervals (SDANN).

Figure 3. Effect of scopolamine and placebo treatments on the ratio of the areas in the low and high frequency bands of the power spectrum (LF/HF). Bars represent mean value ± SEM. *p < 0.05, analysis of variance for repeated measures.
Baroreceptor reflex sensitivity. Analysis of variance for the baroreceptor reflex sensitivity disclosed a significant difference among the three groups (p < 0.001). Similar to the other variables analyzed, no difference was found between control and placebo conditions (p = 0.34). Scopolamine increased baroreceptor reflex sensitivity by 42% compared with control values (from 8.1 ± 4.6 to 11.5 ± 6.4 ms/mm Hg, p = 0.0006). Baroreceptor reflex sensitivity was also significantly greater in the scopolamine test than during placebo conditions (p = 0.01) (Fig. 4). Figure 5 shows the good correlation (r = 0.84, p < 0.001) between baroreceptor reflex sensitivity at baseline and after scopolamine. Figure 5 (right) identifies the patients with increased, and those few with decreased, baroreceptor reflex sensitivity. Figure 6 is an example of the linear regression of two baroreceptor reflex sensitivity tests in one patient during control conditions and after scopolamine.

Correlation of all of the variables studied with the age of the patients was evaluated. This disclosed a moderate negative correlation between age and baroreceptor reflex sensitivity (r = 0.63, p < 0.005) and very modest negative correlations between age and the SD of the mean RR interval, the mean square successive difference and the percent of intervals differing >50 ms from the preceding interval (r = −0.49 to −0.42, p = 0.025 to 0.06). No other significant correlation was found. No correlation was found between ejection fraction and the measures of vagal tone and reflexes. No difference was also found during any study conditions between patients with inferior versus anterior myocardial infarction. However, it is possible that the absence of difference may be due to the relatively small sample size of this study.

In the overall group of patients, scopolamine did not cause significant changes in blood pressure (126 ± 17 vs. 132 ± 18 mm Hg during control conditions for systolic blood pressure). The standard 12-lead ECG disclosed no significant difference in the PR interval (172 ± 28 ms during control conditions and 178 ± 24 ms during the scopolamine test) or in QT interval corrected (QTc) for heart rate (QTc 420 ± 34 before and 412 ± 30 during the scopolamine test). In one patient, Wenckebach second-degree atrioventricular (AV) block occurred during phenylephrine injection in two of four scopolamine tests but in none of the control injections.

An echocardiographic evaluation was performed in nine patients during both placebo and scopolamine administration, and no difference was found in either dimension or function of the left ventricle (ejection fraction was 54 ± 12% during control conditions and 53 ± 10% during scopolamine treatment).

Discussion

This study indicates that transdermal administration of scopolamine significantly increases measures of heart rate variability and baroreceptor reflex sensitivity in patients with a recent myocardial infarction. These markers of vagal tone and reflexes are shifted toward values associated with a lower mortality in previous clinical studies (8–10). Pharmacologic modulation of the autonomic nervous system may thus result in a shift of the sympathovagal balance toward a
vagal dominance and may deserve evaluation as a new approach in the prevention of sudden death after myocardial infarction.

Background. Scopolamine has been shown to have vagomimetic effects in healthy young volunteers (16). However, the possibility of a similar effect in patients after myocardial infarction was uncertain because 1) the age range of the subjects in the study by Dibner-Dunlap et al. (16) (22 to 34 years, mean age 27) was completely different from that of patients with myocardial infarction. Age exerts a considerable influence on markers of vagal activity and specifically on baroreceptor sensitivity (8,9,21); 2) myocardial infarction creates a derangement of the autonomic balance, resulting in an impairment of the parasympathetic component (6,17,18). Thus, the observation in healthy young adults cannot necessarily predict the results in older subjects with myocardial infarction. The finding that scopolamine may also increase heart rate variability and baroreceptor reflex sensitivity in patients shortly after myocardial infarction may have clinical implications because higher values of both markers are correlated with a better prognosis (8–10).

Baseline findings. The baroreceptor reflex sensitivity in this group of patients was very similar to that in a previous study by our group (8). Similar to that study, we found no correlation between baroreceptor reflex sensitivity and ejection fraction, and a moderate negative correlation between age and baroreceptor reflex sensitivity. A modest correlation with age was also found for heart rate variability indexes. The absence of a stronger correlation was expected because the presence of myocardial infarction is likely to perturb the relation between age and vagal effects.

Effects of scopolamine. The mean RR interval increased after scopolamine administration by a value comparable to that (13%) observed in healthy young men by Dibner-Dunlap et al. (16). The increase in SD was also similar to that in the latter study (31%) despite the great difference in baseline values (35 vs. 105 ms). The findings are strengthened in this study by the marked and significant effect of scopolamine on two time domain variables considered as indexes of tonic vagal activity to the heart (22): the mean square successive difference and the percent of intervals differing >50 ms from the preceding interval. Among the patients with a SD of the mean RR interval below the mean value of the whole group (n = 10), 9 of 10 increased their heart rate variability, often considerably. Therefore, the effect of scopolamine appears to be particularly marked among patients considered to be at higher risk.

Frequency domain analysis of heart rate provided a further means to investigate the autonomic regulation of the heart. The ratio of low frequency to high frequency spectral densities has been proposed as an index of sympathovagal balance (23). The decrease in this ratio, observed in the scopolamine test, indicates that the autonomic balance was modified toward vagal predominance.

Vagal reflexes were also significantly increased 24 h after application of the scopolamine patch. Baroreceptor reflex sensitivity increased by 42% compared with control values. More specifically, eight patients with a baseline baroreceptor reflex sensitivity <6 ms/mm Hg showed an average increase in sensitivity of 53%; five patients with a baseline baroreceptor reflex sensitivity <4 ms/mm Hg showed an average increase of 74%; and three patients with a baseline baroreceptor reflex sensitivity <3 ms/mm Hg showed an average increase of 79%. This is an important point in the study because it indicates that the effects of scopolamine were also clearly present in patients considered to be at very high risk (8,9) (i.e., baroreceptor reflex sensitivity <3 ms/mm Hg).
Mechanisms. The first observation that low doses of atropine cause a reduction in sinus rate was made as early as 1915 (14). However, both central (24,25) and peripheral (26) sites of action have been suggested, and a precise explanation for this paradoxic vagomimetic effect is still lacking (27). Relevant here is the fact that, compared with most other muscarinic antagonists, scopolamine has a greater central effect (28).

Characteristics and limitations of the study. Analysis of heart rate variability was performed with relatively short periods of recording so that the assessment could be coupled with that of baroreceptor reflex sensitivity. The result was a total duration of the test of <90 min that would not cause the patient discomfort and thereby alter the results. This relatively short duration makes a study of this type feasible as a clinical tool, at least for selected patients. We decided not to base our study on repeated Holter recordings because this would have doubled the duration of the protocol owing to the lag time for scopolamine absorption and the half-life of the drug (16). Moreover, it was possible to strictly control the recording conditions, including recording time, room temperature and position and activity of the patients, whereas these conditions may vary widely during repeated Holter recordings and have a profound influence on heart rate variability. Finally, stationary signals, such as those obtained during controlled recording in this study, are better suited for both time and frequency domain analysis (29).

For assessment of baroreceptor reflex sensitivity we utilized noninvasive measurements with the digital plethysmographic technique. This technique has been validated for continuous beat to beat blood pressure recording (30). A preliminary analysis of the baroreceptor reflex sensitivity values obtained with intraarterial methods and with Finapres in 376 patients after myocardial infarction, as part of the multicenter ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) study, shows very good correlation (r = 0.92 [31]). On the basis of these results, the use of noninvasive assessment of baroreceptor reflex sensitivity was preferred, also because the measurements were repeated three times. Noninvasive blood pressure measurement is clearly a less stressful condition for the patient compared with intraarterial recording.

The absence of any significant difference between the control and placebo conditions indicates that 1) the effect was indeed due to scopolamine; 2) no relevant spontaneous change in vagal activity occurred during the period of the study; 3) the effect was reversible; and 4) the placebo patch had no effect on the assessed variables. In addition, the present results are in agreement with those of two similar studies performed at the same time by two other groups (32,33).

This study enrolled unselected patients after myocardial infarction. As a consequence, several patients had good ventricular function and would thus be considered at low risk by the traditional risk stratification criteria. However, autonomic variables, notably baroreceptor reflex sensitivity, have no correlation with ejection fraction, and they appear capable of identifying patients at very high risk also among the subgroup with a very good ejection fraction (8,9). We decided to study patients without drugs for this preliminary study, to avoid potentially confounding agents. However, it will be useful to confirm these effects in patients taking their routine drug treatment.

Potential clinical implications. Increasing evidence suggests that vagomimetic interventions may decrease the likelihood of malignant arrhythmia in the setting of acute myocardial ischemia (1–4). Vagal nerve stimulation markedly decreased the incidence of ventricular fibrillation (34) in a conscious canine model for sudden cardiac death that combined a previous myocardial infarction, elevated sympathetic activity and transient myocardial ischemia (11). In the same experimental model, the muscarinic agonists methacholine and oxotremorine provided incomplete but significant protection against sudden death (35). These drugs, at variance with the beta-blocker propranolol, were completely devoid of negative inotropic effects (35). Oxotremorine also markedly decreased the incidence of life-threatening ventricular arrhythmias induced in a feline model by the combination of acute myocardial ischemia and sympathetic hyperactivity (36). Thus, vagomimetic agents may represent an interesting approach, particularly when beta-blockers are contraindicated and when preservation of inotropism is essential.

However, the potential clinical application of muscarinic agonists must await the availability of compounds that display acceptable selectivity for cardiac muscarinic (M2) receptors (27). Pharmacologic modulation of neural activity with scopolamine may effectively increase vagal activity with limited side effects and may thus deserve further evaluation in the search for new strategies in the prevention of sudden cardiac death after myocardial infarction.

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