The long-term increase of baseline and reflexly augmented levels of human vagal-cardiac nervous activity induced by scopolamine

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ABSTRACT We tested the hypothesis that transdermal scopolamine increases vagal-cardiac nervous outflow over the long term in 16 healthy young men. Twenty-four hours after application of one scopolamine patch, the average RR interval was increased by 13% and the average standard deviation of the RR interval (taken as an index of the level of vagal-cardiac nervous activity) was increased by 37%. Baroreceptor-cardiac reflex responsiveness (as reflected by prolongation of RR interval provoked by graded neck suction) also was increased substantially. These findings suggest that vagal-cardiac nervous activity can be augmented pharmacologically in man on a long-term basis. Since vagal outflow influences cardiac electrical properties in an important way, these findings may have therapeutic implications.


A LARGE BODY of basic and clinical research suggests that levels of autonomic nervous system activity contribute to the occurrence of ventricular arrhythmias and sudden cardiac death.1-4 The harmful effects of increased sympathetic activity have been well documented,4 and the beneficial effects of sympathetic blockade in reducing mortality in high-risk patients have been recognized.5-8

Recent attention has been directed toward the electrophysiologic effects of the parasympathetic nervous system.9 Reduced vagal-cardiac activity, as reflected by diminished respiration-related variations in heart rate (respiratory sinus arrhythmia), is associated with an increased incidence of sudden death.10-13 and parasympathetic blockade has been shown to precipitate ventricular arrhythmias, including ventricular fibrillation.14 In contrast, augmentation of vagal-cardiac activity provoked by injections of pressor drugs or carotid sinus massage may terminate ventricular tachycardia in man.15, 16

The possibility that vagal-cardiac nervous activity could be augmented over the long term in man has not been tested. However, we demonstrated recently that cardiac parasympathetic outflow could be increased over the short term in man with low-dose atropine.17, 18 We therefore conducted the present study to determine if transdermal scopolamine (Transderm-Scop, CIBA Consumer Pharmaceuticals, Edison, NJ) would augment vagal-cardiac activity over the long term. Our results indicate that transdermal scopolamine does substantially increase baseline and reflexly augmented levels of cardiac parasympathetic activity over the long term in normal man.

Methods

We studied 16 normal male volunteers whose average age was 27 ± 4 (± SEM; range 22 to 34) years. All volunteers gave their written informed consent for the study. All were in good health according to histories and results of physical examinations; none were smokers and none took medications or caffeine-containing beverages immediately before or during the study. Volunteers were studied while supine at quiet rest; their usual activity was reading. Surface electrocardiograms were recorded on electrostatic and FM tape recorders. Blood pressure was monitored with a sphygmomanometer.

Electrocardiographic analyses. Vagal-cardiac activity was assessed from RR intervals and standard deviations of RR intervals. Standard deviations of RR intervals provide accurate, noninvasive indexes of cardiac parasympathetic neural activity.19, 20 RR intervals and their standard deviations were
measured over 28 min time intervals by a computer in real-time or batch modes. Two 28 min data sets were combined to obtain 56 min averages (to simplify data presentation), according to the following formulas:

\[
\text{Mean RR interval} = \frac{(n_1)(M_1) + (n_2)(M_2)}{n_1 + n_2}
\]
\[
\text{Mean SD} = \frac{(n_1 - 1)SD_1^2 + (n_2 - 1)SD_2^2}{n_1 + n_2 - 2}
\]

where \( n \), \( M \), and \( SD \) refer to the number of RR intervals in the time interval, their mean intervals, and their standard deviations, and the subscripts 1 and 2 refer to the first and second 28 min periods. Continuous electrocardiographic recordings of the electrocardiogram were scanned visually, and premature atrial and ventricular beats and the intervals preceding and following them were excluded from analyses. All subjects were in sinus rhythm.

**Analysis of scopolamine levels.** Plasma scopolamine levels were measured in the Research and Clinical Laboratories, Johnson Space Center, with a recently developed, reversed-phase liquid chromatography and radioreceptor assay.\(^\text{21}\) This method detects scopolamine levels as low as 25 pg/ml, and has a coefficient of variation of less than 12%. We analyzed measured plasma scopolamine levels with Marquardt-Levenberg curve fitting on the Prophet Computer System of the National Institutes of Health, and derived estimates of plateau concentrations, first-order rate constants, and lag times from a modified one-compartment, continuous-infusion model.\(^\text{22}\)

**Protocol.** This study was conducted in three sequential phases to determine: (1) the time course of action of transdermal scopolamine, (2) the dose required to produce the maximal parasympathomimetic effect, and (3) the degree of baseline and reflexly augmented vagal-cardiac activity produced at the optimal time and dose.

In phase 1 in seven subjects we measured the time course of electrocardiographic changes and changes in plasma scopolamine levels after application of one patch of scopolamine. Baseline electrocardiographic measurements were obtained for 1 hr, beginning at 7 A.M., and then one patch of transdermal scopolamine was applied to the skin behind one ear of each subject. The electrocardiogram was recorded continuously for an additional 12 hr (two half-hour breaks were allowed for meals), and subjects returned the following morning, with the patch still in place, for an additional hour of measurements. Blood pressure was measured hourly. Blood was drawn for determination of scopolamine levels at hourly intervals for 12 hr, and at 24 hr. Similar electrocardiographic and blood pressure measurements were made in five volunteers before and after application of placebo patches (supplied by CIBA Consumer Pharmaceuticals, Edison, NJ). In eight additional subjects, electrocardiograms were recorded before and 24 hr after application of one placebo or one scopolamine patch.

In phase 2 of the study we determined the effects of different doses of transdermal scopolamine on mean RR interval and its standard deviation in six volunteers. These subjects were given a half, one, or one and a half scopolamine patches, and electrocardiograms were obtained before and 24 hr after application of the drug.

In phase 3 we compared responses to one scopolamine patch with those to one placebo patch in a randomized, single-blind, crossover experiment in eight subjects. In this study, we also measured sinus node responses to baroreceptor stimulation with graded neck suction.\(^\text{23}\) Seven stimuli of –15, –30, and –45 mm Hg each were applied for 0.6 sec during held expiration, and were timed to begin 0.75 to 0.85 sec before the expected occurrence of the next P wave, as described previously.\(^\text{24}\) These measurements were made before application of one patch (scopolamine or placebo) and 24 hr later.

**Statistical analysis.** Results are expressed as mean ± SEM. Normality was demonstrated with the Wilk-Shapiro test and homogeneity of variance was established with Levene's test. Statistical comparisons were made with least squares linear regression, Student's paired t test, analysis of variance, and the Student–Newman-Keuls test for analysis of covariance.\(^\text{25}\) A p value of less than .05 was considered indicative of significance.

**Results**

**Plasma scopolamine levels.** Measured plasma scopolamine levels 24 hr after application of the transdermal patch and derived plateau scopolamine concentrations, first-order rate constants, and lag times for seven subjects are listed in table 1. The average measured scopolamine level 24 hr after application of the transdermal patch was 143 ± 20 pg/ml. The calculated mean plateau scopolamine concentration was 156 ± 20 pg/ml, and the first-order rate constant was 0.17 ± 0.03/hr. These data led to an estimate of 4.1 hr for the biologic half-life of the drug in these seven subjects. The mean lag time between application of the patch and the detection of scopolamine in the systemic circulation was 3.6 ± 0.4 hr.

**Time course of changes in RR intervals.** Changes of cardiac cycle length were evaluated before and after application of one scopolamine patch in 14 individuals. Sequential electrocardiographic data could be analyzed reliably in six of the seven subjects for whom serial scopolamine levels were obtained. In one subject frequent premature atrial beats occurred after application of the patch, rendering accurate assessment of his

| TABLE 1 | Plasma scopolamine measurements |
|---|---|---|---|---|---|
| Subject No. | Scopolamine level (pg/ml) | C₀ (pg/ml) | k (per hour) | Lag time (hr) | p value |
| 1 | 223 | 235 | 0.20 | 4 | <.0001 |
| 2 | 204 | 218 | 0.17 | 4 | <.0001 |
| 3 | 94 | 111 | 0.09 | 2 | <.0001 |
| 4 | 95 | 128 | 0.06 | 3 | <.0005 |
| 5 | 158 | 178 | 0.20 | 3 | <.0001 |
| 6 | 108 | 107 | 0.25 | 5 | <.0001 |
| 7 | 120 | 117 | 0.23 | 4 | <.0001 |
| Mean | 143 | 156 | 0.17 | 3.6 | |
| SEM | 20 | 20 | 0.03 | 0.4 | |

Measured plasma scopolamine levels (C₀) 24 hours after application of one transdermal patch, and plateau scopolamine concentrations (Cₑ) and first-order rate constants (k) for seven subjects, calculated from a one-compartment, continuous administration model\(^\text{22}\) with the equation C = Cₑ(1 – e⁻kt), modified by the addition of a lag time. The p values refer to the correspondence between the model and measured plasma scopolamine levels.
electrocardiogram changes impossible. (The responses of this patient to scopolamine are discussed in detail below.)

Plasma scopolamine levels, RR intervals, and standard deviations of RR intervals for one volunteer are shown in figure 1. Both RR intervals and standard deviations tended to increase in parallel with increases in plasma scopolamine levels. RR interval and its standard deviation were initially below baseline levels in this volunteer at the lowest measurable scopolamine plasma level. In each of the six subjects, the RR interval, or its standard deviation, or both were slightly diminished compared with baseline at the lowest measurable scopolamine level.

In eight additional subjects, RR intervals and standard deviations of RR intervals were measured before and 24 hr after application of one scopolamine patch. Responses in all 14 subjects are summarized in figure 2. The average RR interval increased 13%, from 0.974 ± 0.037 sec (62 beats/min) to 1.104 ± 0.034 sec (54 beats/min) after scopolamine (p = .001), and the standard deviation of the RR interval increased 31%, from 0.105 ± 0.009 to 0.138 ± 0.012 sec (p = .002). It is likely that the increases in RR interval and standard deviation of RR interval that we observed were due to scopolamine rather than to other factors, since in five subjects studied twice (with neither placebo nor scopolamine patches applied between studies) and in eight subjects studied before and 24 hr after application of placebo patches the average RR interval and standard deviation of RR interval did not change significantly. Average systolic and diastolic blood pressures were 115 ± 4 and 74 ± 2 mm Hg before application of one scopolamine patch and were unaltered (114 ± 3 and 71 ± 4 mm Hg) 24 hr after one scopolamine patch was applied (p = .364 and p = .467).

There was a loose (r = .58) but significant (p = .030) association between changes in RR interval and changes of standard deviation of RR interval. In two subjects both RR interval and standard deviation declined (figure 2), and in 12 subjects both parameters increased after scopolamine. There was no significant relationship between changes in standard deviation of RR interval and baseline levels (p = .590); thus, volunteers with the lowest baseline levels were as likely to have large increases in standard deviations after scopolamine as volunteers with the highest baseline levels. The two volunteers who experienced reductions in standard deviations after scopolamine (figure 2) could not be distinguished from the others on the basis of their baseline standard deviations.

The electrocardiogram, RR intervals, and frequency distributions of RR intervals for one subject are shown in figure 3. This subject had the lowest baseline stan-
standard deviation of RR interval of all subjects studied. His average RR interval increased from 0.839 to 1.062 sec (27%), and his average standard deviation of RR interval increased from 0.051 to 0.113 sec (122%) 24 hr after the scopolamine patch was applied. In this subject, increases in the dispersion of RR intervals resulted primarily from addition of longer periods (figure 3, right); the shortest periods in the frequency distribution were nearly comparable before and after scopolamine. It is highly likely that this large increase in standard deviation was due to scopolamine rather than spontaneous variability since in this subject baseline standard deviations were measured on 4 different days and were consistent and small (averaging 0.051, 0.043, 0.057, and 0.053 sec).

**Dose-ranging studies.** The effects of a half, one, and one and a half scopolamine patches on RR intervals and standard deviations of RR intervals in six subjects are illustrated in figure 4. A half patch of scopolamine did not significantly alter mean RR interval (p = .815) or standard deviation of RR interval (p = .638). One patch of scopolamine, however, provoked significant increases of both mean RR interval and standard deviation of RR interval (both p < .001) in these six subjects. Although average RR intervals were increased significantly (p = .021) 24 hr after application of one and a half scopolamine patches, no significant change was apparent for standard deviation of RR interval (p = .265). Since the one-patch dose of scopolamine caused the maximum increase in standard deviation of RR interval, this dose was selected for the phase 3 study.

**Baroreceptor-cardiac reflex responses.** The effect of one patch of scopolamine on baroreceptor stimulation with graded neck suction is shown in figure 5. Barore-

**FIGURE 3.** RR intervals and electrocardiogram of one volunteer before (top) and 24 hr after (bottom) application of one scopolamine patch. Frequency distributions of RR intervals are shown on the right.

**FIGURE 4.** Changes in RR intervals and standard deviations of RR intervals after application of a half, one, and one and a half scopolamine patches. Differences between baseline observations and those obtained 24 hr after the application of scopolamine patches are shown as percent changes ± SEM. Statistical comparisons of responses to drug doses with baseline observations were made with the Student's paired t test: *p < .05; **p < .001.
levels of vagal-cardiac nervous traffic over the long term in man. Since the level of autonomic nervous outflow to the heart influences cardiac electrophysologic properties in an important way, our findings may have therapeutic implications.

It has been well established that small doses of anticholinergic drugs cause paradoxic Bradycardia,26, 27 an effect that is attributed to influences exerted in the central nervous system (see below). Since scopolamine is believed to provoke greater central reactions than other anticholinergic drugs,28 we predicted that its long-term, low-dose administration by a transdermal delivery system29 would be associated with vagomimetic effects similar to those observed after small intravenous doses of atropine sulfate.17, 18, 30 Our study validates this hypothesis and provides additional new information on the time course of plasma levels and electrocardiographic effects after application of scopolamine, the dose required to provoke the maximal vagal-cardiac effects, and the magnitude of those effects.

**Plasma scopolamine levels.** Measurements of plasma levels of scopolamine after transdermal application of one scopolamine patch documented an approximate 3.6 hr lag between the application of the patch and the detection of scopolamine in the systemic circulation. This may have resulted from delays associated with diffusion through the epidermis and saturation of skin binding sites.31

**Time course of effects of scopolamine.** RR intervals and their standard deviations appeared to increase in parallel with increases in plasma levels of scopolamine. However, the lowest measurable scopolamine level was associated with a slight reduction in the RR interval and its standard deviation. Subsequent increases in plasma drug levels tended to be reflected by parallel increases in RR intervals and standard deviations, and the highest plasma levels were associated with the highest average RR intervals and standard deviations. The apparent biphasic effect (and major reductions in RR intervals and standard deviations that occurred in two subjects after application of a half patch of scopolamine) probably results from scopolamine rather than from other factors, since List and Gravenstein27 found similar biphasic RR interval responses to incremental small intravenous doses of the drug.

We focused on responses at 24 hr because the preliminary data we obtained during the first phase of this study showed that changes in RR interval and its standard deviation were appreciable and significant at this time. We assume that increases in RR interval and its standard deviation would have continued beyond 24

### Discussion

Our findings suggest that transdermal scopolamine, a preparation used widely for prevention of motion sickness, increases baseline and reflexly augmented...
hr, because cardiac slowing persists for at least 48 hr after application of transdermal scopolamine.*

**Dose-ranging studies.** One scopolamine patch provoked significant increases in RR interval and its standard deviation, and this dose was used in phase 3 studies of baroreflex responses (figure 4). Although one and a half patches provoked average RR interval lengthening similar to that caused by one patch, no significant change in the standard deviation of RR interval was apparent; moreover, this dose was unacceptable because of the side effects it produced. The increased frequency of premature atrial beats in one subject may have been related to scopolamine, since augmented levels of vagal-cardiac traffic are known to predispose to atrial arrhythmias,32 and in this subject, the number of premature atrial beats appeared to be dose related.

**Site of vagomimetic action of transdermal scopolamine.** It is highly likely that scopolamine exerts its primary effect on the RR interval and its standard deviation by altering the firing frequency of medullary vagal-cardiac motoneurones. First, the standard deviation of RR interval increased substantially in 12 of 14 subjects after scopolamine (figure 2). Katona and Jih19 found in anesthetized dogs that respiration-related changes in peak-valley cardiac cycle length provided nearly linear estimates of vagal-cardiac activity, as judged from the shortening of RR intervals that occurred after vagal cold block. Fouad et al.33 also measured peak-valley RR interval changes in healthy human volunteers and found that these measurements corresponded closely with the shortening of RR intervals that occurred after large intravenous doses of atropine sulfate. (We used standard deviation in the present study because a previous study showed that standard deviation was a linear function [r = .97] of peak-valley RR intervals,20 and standard deviation measurements lend themselves to automated computer analysis.) Second, low doses of atropine sulfate given intravenously to dogs34 or applied iontophoretically directly to medullary vagal-cardiac motoneurones in cats35 increase vagal-cardiac motoneuron activity.

**Augmentation of baroreceptor-cardiac reflexes.** Transdermal scopolamine significantly increased abrupt RR interval prolongations caused by carotid baroreceptor stimulation with neck suction (figure 5). The greatest percentage increase (78%) occurred after the smallest baroreceptor stimulus used was applied. This change may have substantial physiologic significance, since in resting man, arterial baroreceptors modulate muscle sympathetic and vagal-cardiac outflow over very narrow arterial pressure ranges, and small changes in blood pressure elicit important adjustments of autonomic neural outflow.† (Although we have focused on abrupt, vagally mediated cardiac cycle length changes, we have not excluded the possibility that scopolamine may also modify sympathetic cardiovascular reflex responses.) We doubt that the augmentation of sinus node responses we observed after scopolamine was specific for arterial baroreflexes, since low intravenous doses of another anticholinergic agent, atropine sulfate, have been shown to augment bradycardia provoked by trigeminal nerve stimulation with face immersion.18 Moreover, it is likely that scopolamine potentiates other vagal-cardiac reflex responses, including those that accompany inferior myocardial ischemia.36

**Potential therapeutic implications.** It is widely held that the occurrence of ventricular fibrillation depends in an important way on levels of autonomic neural outflow to the heart, such that increased levels of sympathetic activity predispose to ventricular fibrillation and increased levels of parasympathetic activity protect against it.30,37 Large clinical trials of patients who have suffered myocardial infarction show that β-adrenergic blockade significantly reduces the incidence of sudden cardiac death.5,6,8,38 Recent studies by Billman et al.39 emphasize the adverse prognostic implications of reduced levels of vagal-cardiac nervous activity: In dogs that have recovered from acute myocardial infarction, the likelihood of occurrence of exercise-induced ventricular fibrillation is inversely related to their vagal baroreceptor-cardiac reflex responsiveness at rest.39 Moreover, in these dogs, subsequent increases in baroreflex responsiveness (resulting from exercise, or occurring spontaneously) appear to protect against ventricular fibrillation.40

Results from a new collaborative study also underscore the potential importance of vagal-cardiac nervous outflow. These data, reported in preliminary form,13 demonstrate that the standard deviation of averaged RR intervals (a measure of heart rate variability) provides important prognostic information in post-infarction patients. In 819 patients, low standard deviations of averaged RR intervals predicted subsequent mortality better than more widely used systems for grading the severity of ventricular arrhythmias. Moreover, this measure appeared to yield prognostic information that was independent of that provided by ventricular function studies.▼ This study and the post-

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infection studies in dogs cited above\textsuperscript{39, 40} suggest that an intervention that increases vagal-cardiac nervous activity may improve the prognosis of carefully selected, high-risk postinfarction patients.

Although the electrocardiographic changes we observed after transdermal scopolamine were moderate (average RR intervals and standard deviations increased by 13\% and 31\%; figure 2), it is highly likely that they substantially altered cardiac electrical properties. Antzelevitch et al.\textsuperscript{41} have shown that in isolated dog Purkinje fibers, very small changes in pacemaker rate may alter cardiac electrical properties profoundly, and Winkle\textsuperscript{42} has shown that, in cardiac patients, minor cardiac slowing may be associated with striking reductions in the frequency of ventricular premature beats. We recently showed that a low intravenous dose of atropine sulfate (2 \mu g/kg) reduced vulnerability to ventricular fibrillation in conscious dogs with normal or acutely ischemic hearts.\textsuperscript{30}

In summary, our data suggest that transdermal scopolamine increases resting and baroreflex-augmented levels of vagal-cardiac nervous activity. Our study was not designed to determine if scopolamine should be used therapeutically in patients with heart disease. However, our data suggest that vagal-cardiac nervous activity can be increased over the long term in man, and this possibility has not been recognized before.

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