Assessment of Effects of Autonomic Stimulation and Blockade on the Signal-Averaged Electrocardiogram

Jeffrey J. Goldberger, MD; Mirza W. Ahmed, MD; Michele A. Parker, RN, MS; Alan H. Kadish, MD

Background Signal-averaged ECG is a noninvasive test designed to detect “late potentials.” The effects of alterations in autonomic tone on the signal-averaged ECG have not been evaluated systematically.

Methods and Results The effects of autonomic stimulation and blockade on the signal-averaged ECG were evaluated in 14 healthy subjects (8 men and 6 women; age, 28.5 ± 4.8 years) on 2 separate days. The signal-averaged ECG was recorded at baseline and after physiological and pharmacologic β-adrenergic stimulation (tilt, exercise, and epinephrine and isoproterenol infusions), sequential and combined β-adrenergic and parasympathetic blockade, and α-adrenergic stimulation before and after parasympathetic blockade. Analysis was performed with a bidirectional filter (40-Hz high-pass). Significant changes in the signal-averaged QRS duration from baseline (105.1 ± 12.0 milliseconds) were noted with tilt (96.8 ± 8.8 milliseconds), tilt after double blockade (97.5 ± 9.0 milliseconds), epinephrine (110.5 ± 11.8 milliseconds), and isoproterenol (99.6 ± 12.6 milliseconds). Changes in the root-mean-square voltage of the terminal 40 milliseconds and the low-amplitude (<40 μV) signal duration paralleled the changes in the QRS duration.

Conclusions The signal-averaged ECG does not measure only “fixed” parameters but rather is altered under a variety of physiological and pharmacologic conditions. Upright tilt leads to shortening of the QRS duration before and after autonomic blockade; thus, the decrease in QRS duration with tilt may be related to factors other than changes in autonomic tone. These findings have implications for interpretation of the results of signal-averaged ECG. (Circulation. 1994;89:1656-1664.)

Key Words • QRS duration • catecholamine • tilt • electrocardiography

The signal-averaged ECG was initially developed to allow recording of low-amplitude ventricular depolarizations on the surface ECG in patients with myocardial infarctions who are susceptible to ventricular tachycardia. These low-amplitude depolarizations, called “late potentials,” correspond to delayed and fragmented endocardial or epicardial electrograms and are believed to represent a “fixed” substrate for reentrant ventricular tachycardia in these patients. The effects of alterations in autonomic tone on the signal-averaged ECG have not been evaluated systematically in either healthy or diseased populations. Such alterations may be important because autonomic tone is frequently altered in patients to whom the signal-averaged ECG has been applied, such as those who have had a myocardial infarction.

Although the signal-averaged ECG was originally developed for use in patients with coronary artery disease and ventricular tachycardia, it has subsequently been applied to a variety of other groups of patients, most notably those with syncope and dilated cardiomyopathy. It has been proposed to have prognostic significance in these patients, but the pathophysiological basis for this finding has not been explored. Because autonomic tone has also been shown to be an important prognostic factor in some of these subgroups, we postulated that changes in autonomic tone may affect the signal-averaged ECG and perhaps contribute to its prognostic value in patients without coronary artery disease.

To evaluate the potential effects of autonomic tone on the signal-averaged ECG, we initially chose to perform a comprehensive evaluation of autonomic stimulation and blockade on the normal signal-averaged ECG. Although we considered including subjects with myocardial infarctions and late potentials in the present study, we did not believe that we could ethically recruit an adequate number of patients who could undergo the entire protocol.

Methods

Subjects

Fourteen healthy volunteers (8 men and 6 women; age range, 22 to 38 years; mean age, 28.5 ± 4.8 years) were studied at the Clinical Research Center at Northwestern Memorial Hospital. These subjects were also included in a separate study of the effects of autonomic manipulation on heart rate variability. Subjects were instructed to abstain from smoking or coffee consumption for 24 hours before the study. No subject had a history of diabetes mellitus, hypertension, syncope, palpitations, or cardiac disease. All subjects had a normal physical examination, a normal resting ECG, a normal hematocrit, and normal serum electrolytes. No subjects were taking medications. Written, informed consent was obtained before the study. The study was approved by the Northwestern University Institutional Review Board.
Data Acquisition

The study was performed on 2 separate days. Subjects were admitted to the Clinical Research Center in the morning after an overnight fast. An indwelling intravenous catheter was placed in the forearm, through which normal saline was infused at a rate of 30 mL/hr. A blood sample was withdrawn for measurement of serum electrolytes. Subjects were attached to a cardiac monitor and an automated blood pressure device (Accutrack-Marquette Electronics). In male patients, the chest hair was shaved as needed for proper electrode attachment. After thorough cleansing and mild abrasion of the skin, Ag-AgCl adhesive electrodes were applied as X leads in the right and left axilla, Y leads at the manubrium sterni and the left upper abdomen, and Z leads at the fifth intercostal space in the midclavicular line and the opposite point in the posterior chest.

ECG data were recorded on a commercially available system (Corazonitor Predictor 1) via the X, Y, and Z leads. ECG recordings were made at a sampling frequency of 1000 Hz and stored on an optical disk for subsequent analysis. All recordings were made in 5-minute segments.

Study Design

The study was designed to evaluate the effects of β-adrenergic, α-adrenergic, and parasympathetic stimulation as well as the effects of β-adrenergic and parasympathetic blockade on the signal-averaged ECG. Recordings were made in each subject during rest conditions and after a total of 10 interventions. Four different sympathetic stimuli were evaluated. Upright tilt and exercise were selected as standard physiological maneuvers that enhance adrenergic tone. To evaluate whether the effects observed with tilt were related to increased sympathetic tone or to the change in position, tilt was repeated in the presence of β-adrenergic and parasympathetic blockade. Iso- proterenol infusion was chosen as a test condition to evaluate the effects of circulating catecholamines, in particular, pure β-adrenergic stimulation. Finally, epinephrine infusion was also tested to determine the effects of a naturally occurring circulating catecholamine. Assessment of the effects of sequential and combined β-adrenergic and parasympathetic blockade was performed by administration of propranolol and atropine. Increased parasympathetic tone may be achieved via baroreflex activation using phenylephrine. Thus, phenylephrine was infused to achieve a 20 to 30 mm Hg increase in systolic blood pressure. To assess the effect of isolated α-adrenergic stimulation, we infused phenylephrine after administration of atropine, which blocks the baroreceptor-mediated increase in parasympathetic tone.

Clinical Protocol

Baseline 1

On day 1, subjects were allowed to rest comfortably in the supine position in a quiet room with dim lights. After the subjects had rested for 15 minutes, four baseline 5-minute recordings were obtained.

Sympathetic Stimulation

Tilt. Before tilt and after supine rest by subjects for approximately 30 minutes, blood was withdrawn via the indwelling intravenous catheter for measurement of plasma catecholamine levels (n=13; samples inadequate from 1 subject). Subjects were then tilted to 70° on an electrically driven tilt table with a foot support while the heart rate and blood pressure were monitored. After 5 minutes of tilt, three ECG recordings were made during tilt. Premature termination of the tilt test was required in 4 subjects because of nausea and/or lightheadedness. However, at least one complete recording was obtained in all except 1 subject. After data acquisition, blood for determination of plasma catecholamine levels was again obtained (n=9); subjects were then returned to the supine position, and heart rate and blood pressure were allowed to return to baseline.

Epinephrine infusion. Epinephrine infusion was then begun at a rate of 2 μg/min with subjects in the supine position and resting comfortably. The dose was gradually increased in all subjects to 50 ng·kg⁻¹·min⁻¹ and maintained at this infusion rate for 10 minutes to achieve steady state. Previous studies have demonstrated that a steady-state concentration is achieved after 10 minutes of infusion. This infusion rate was used because it has been shown to result in elevation of the plasma epinephrine levels similar to that achieved with moderate exercise, hypoglycemia, and acute myocardial infarction. Five-minute ECG recordings were obtained in triplicate, after which the infusion was stopped; serum potassium levels were determined at the end of the infusion. Potassium levels decreased from a baseline of 4.0±0.3 mEq/L to 3.4±0.4 mEq/L; in the 3 subjects with potassium levels of 3.0 mEq/L or less, repeat determinations after washout of epinephrine demonstrated normalization of the potassium level (4.2±0.3 mEq/L). Heart rate and blood pressure were allowed to return to baseline values.

Isoproterenol infusion. Isoproterenol infusion was then started at a rate of 2 μg/min and gradually increased to 50 ng·kg⁻¹·min⁻¹. This infusion rate was maintained for 10 minutes to achieve steady state because it has been shown that at least 90% of the heart rate increase during isoproterenol infusion is achieved by 8 minutes. One subject did not tolerate the maximal dose, and recordings were made at a reduced dose of 25 ng·kg⁻¹·min⁻¹. During the infusion, ECG recordings were made in triplicate. The infusion was then stopped, and time was allowed for heart rate and blood pressure to return to baseline values.

Exercise. Subjects then performed symptom-limited exercise on a bicycle ergometer with progressively increasing workloads. Subjects started at a workload of 150 to 300 kilopond (Kp)·m⁻¹·min⁻¹ (corresponding to approximately 25 to 50 W), which was increased by 150 to 300 Kp·m⁻¹·min⁻¹ every 2 minutes. All subjects exercised to fatigue (mean exercise time, 8.4±2.4 minutes), achieving a maximal heart rate between 150 and 170 beats per minute (mean, 168±12 beats per minute). They were then returned to bed, and after patients had a 2-minute recovery period, we obtained recordings in triplicate. A rest period was allowed for heart rate and blood pressure to return to baseline values.

Blockade

β-Adrenergic blockade. To achieve β-blockade, we administered propranolol intravenously at a rate of 1 mg/min to a total dose of 0.2 mg/kg with subjects in the supine position. After the infusion was completed, three recordings were obtained.

β-Adrenergic and parasympathetic blockade (double blockade). Atropine (0.04 mg/kg) was then administered intravenously over 3 minutes in the presence of β-adrenergic blockade, and three recordings were obtained.

Tilt after double blockade. Because of the elapsed time since the initial administration of propranolol, an additional 0.07 mg/kg propranolol was administered as before. Subjects were then tilted to 70° while heart rate and blood pressure were monitored. After 5 minutes of tilt, three ECG recordings were made during tilt. Again, premature termination of the tilt test was required in 4 subjects because of nausea, lightheadedness, or both. However, at least one complete recording was obtained in all except 1 subject. Blood for determination of plasma catecholamine levels was obtained just before tilt and after the final recording, as described previously.

Baseline 2

Ten of the 14 subjects returned for the second day of the study (2 were no longer available, 1 had been started on a tricyclic antidepressant, and 1 chose not to continue). Subjects were prepared as on day 1 and allowed to rest comfortably in
the supine position in a quiet room with dim lights. After the subjects rested for 15 minutes, four baseline 5-minute recordings were obtained.

Parasympathetic and α-Adrenergic Stimulation

**Phenylephrine infusion.** An intravenous infusion of phenylephrine was begun at 0.2 μg·kg⁻¹·min⁻¹ and gradually titrated to achieve an increase in systolic blood pressure of 20 to 30 mm Hg. The infusion was then maintained at the final dose, during which recordings were made in triplicate. The mean dosage required to achieve this increase in blood pressure was 1.1±0.6 μg·kg⁻¹·min⁻¹. A washout period was then allowed for heart rate and blood pressure to return to baseline values.

**Parasympathetic blockade.** Atropine was administered at a dose of 0.04 mg/kg¹² over a period of 2 minutes. ECG recordings were then obtained in triplicate.

**Phenylephrine infusion after parasympathetic blockade.** A phenylephrine infusion was repeated in the presence of atropine to assess the direct effects of α-adrenergic stimulation on the signal-averaged ECG. The infusion was begun at a rate of 0.2 μg·kg⁻¹·min⁻¹ and titrated to achieve an increase in systolic blood pressure of 20 to 30 mm Hg. The mean dosage required to achieve this increase in blood pressure was 0.3±0.2 μg·kg⁻¹·min⁻¹. ECG recordings were then obtained in triplicate.

Signal-Averaged ECG Analysis

The acquired ECG data were analyzed using the PREDICTOR I software (version 6.0, Corazonix Corp). With a template-matching algorithm, normal QRS complexes in each 5-minute recording were averaged until the residual noise level was 0.2 μV or the end of the recording was reached. The individual X, Y, and Z leads were combined into a vector sum (VX²+VY²+VZ²). Bidirectional filters were used with a bandpass of 40 to 250 Hz.

Automated analysis calculated the following parameters: QRS duration, root-mean-square voltage in the terminal 40 milliseconds of the QRS complex (RMS), and low-amplitude (<40 μV) signal duration (LAS).

All tracings were visually overread, but none required adjustments in the automatically determined QRS onset or offset.

Plasma Catecholamine Levels

Blood samples were collected in heparinized tubes and placed on ice. After centrifugation at 4°C at 3000 rpm for 15 minutes, 2 mL of plasma was transferred to a tube containing 4 mg of reduced glutathione as a preservative and stored at −70°C for subsequent analysis. Catecholamine levels in plasma were assayed by liquid chromatography with electrochemical detection. The method combined liquid-liquid extraction of catecholamines from plasma with reversed-phase chromatography incorporating a cation-exchange reagent (Plasma Catecholamine Kit, Bioanalytical Systems, Inc.²⁰,²¹ For replicate determinations of a 1.0-mL plasma sample

![Fig 1. Facing page. Signal-averaged ECG tracings obtained from a single patient for each condition studied are displayed. Numbers indicate the computer-detected QRS onsets and offsets. Arrow indicates the point from which the low-amplitude signal duration was calculated. Shaded part indicates the area included in the root-mean-square voltage of the terminal 40 milliseconds. At baseline, the QRS duration was 90 milliseconds and shortened to 79 milliseconds and 78 milliseconds with tilt and tilt after double blockade, respectively. With epinephrine infusion, it prolonged to 96 milliseconds, whereas with isoproterenol infusion and exercise, it shortened to 78 milliseconds and 86 milliseconds, respectively. With β-blockade, the QRS duration was 93 milliseconds. With atropine and double blockade, it shortened to 85 milliseconds and 88 milliseconds, respectively. During phenylephrine infusion, the QRS duration was 89 milliseconds, and during phenylephrine infusion after atropine, it was 85 milliseconds.](http://circ.ahajournals.org/)
containing 0.25 pmol epinephrine and 1.6 pmol norepinephrine, intra-assay coefficients of variation were 8% and 1%, respectively, and interassay coefficients of variation were 12% and 4%, respectively.

Data Analysis

The reproducibility of RR intervals at each phase of the study was assessed to determine whether systematic variation related to each intervention was present. Four RR interval measures were made during each of the baseline periods on days 1 and 2; three measures were made for all other conditions. Reproducibility was evaluated by calculating intraclass correlation coefficients using a one-way random-effects ANOVA model.\textsuperscript{22,23} The intraclass correlation coefficient is an assessment of the intrasubject variability of a measure and reflects its reproducibility. An intraclass correlation coefficient of more than 0.8 indicates excellent reproducibility. Values between 0.6 and 0.8 reflect good reproducibility. Values of less than 0.6 indicate fair to poor reproducibility.

Data are presented as mean±SD. Differences in mean values between conditions were assessed using a one-factor ANOVA with repeated measures; the Bonferroni method was used to adjust for multiple comparisons and calculation of 95% confidence intervals. The signal-averaged ECG parameters were compared between the following conditions: baseline on day 1 versus tilt, epinephrine infusion, isoproterenol infusion, exercise, \( \beta \)-adrenergic blockade, and double blockade; tilt versus tilt after double blockade; baseline on day 2 versus phenylephrine infusion; and parasympathetic blockade versus phenylephrine infusion after parasympathetic blockade. All tests are two-tailed; a value of \( P < .05 \) was considered statistically significant.

Results

There were no significant differences among the RR intervals at the two baseline periods (day 1, 980±138 milliseconds; day 2, 1034±109 milliseconds) or after \( \beta \)-adrenergic blockade (921±83 milliseconds). \( \beta \)-Adrenergic stimulation with tilt, epinephrine infusion, isoproterenol infusion, and exercise resulted in expected significant decreases in the RR intervals to 693±62 milliseconds, 760±118 milliseconds, 489±32 milliseconds, and 637±79 milliseconds, respectively, which is consistent with their \( \beta \)-adrenergic effects. The RR interval also shortened with atropine (542±58 milliseconds), atropine plus propranolol (601±42 milliseconds), tilt with double blockade (617±37 milliseconds), and atropine plus phenylephrine (547±77 milliseconds). Phenylephrine infusion resulted in a significant lengthening of the RR interval to 1355±143 milliseconds, which is consistent with a baroreflex-mediated increase in parasympathetic tone. Mean blood pressure was significantly elevated from baseline during both infusions of phenylephrine and after exercise, whereas no significant changes were noted in mean blood pressure with the other conditions (Table 1). The intraclass correlation coefficients for the RR intervals were excellent (≥0.90) at baseline and for all conditions tested except tilt and exercise; thus, for purposes of analysis, the parameters derived from the signal-averaged ECGs for each of these conditions were averaged. The intraclass correlation coefficient using all three RR interval recordings during tilt was 0.72, which was significantly lower than that for the other conditions. However, the intraclass correlation coefficient for the two recordings made at 10 and 15 minutes of tilt was 0.92, indicating that a stable RR interval was not obtained until the last two recordings. In contrast, the intraclass correlation coefficient for all three tilt recordings after double blockade was 0.95. Thus, only data from the last two recordings made during tilt (without double blockade) were averaged for analysis. Finally, the intraclass correlation coefficients for the three postexercise period RR intervals also demonstrated less reproducibility (0.67), and therefore each postexercise recording was analyzed separately.

Signal-Averaged ECG

Typical signal-averaged ECGs recorded during each condition are shown in Fig 1. Table 1 summarizes the results for the signal-averaged ECG findings for all conditions tested. Generally, when the QRS duration shortened, the RMS increased, and the LAS decreased. This effect is shown in Fig 2 for all conditions. Pearson’s correlation coefficient for the RMS with the QRS duration was −0.72 (\( P < .001 \)) and for the LAS with the QRS duration was 0.72 (\( P < .001 \)). Therefore, subsequent results and discussion focus on the QRS duration. There were no significant differences between baseline signal-averaged ECG parameters on day 1 and those on day 2. The intraclass correlation coefficients for the recordings made on the 2 days were 0.96 for the QRS duration, 0.97 for the RMS, and 0.92 for the LAS.

Effects of \( \beta \)-Adrenergic Stimulation

Fig 3 depicts the individual changes in QRS duration noted with tilt, epinephrine infusion, isoproterenol infusion, and after exercise. Upright tilt shortened the QRS duration and epinephrine increased the QRS duration in all subjects. In contrast to epinephrine, isoproterenol shortened the QRS duration in most subjects. Tilt resulted in a significant decrease in the QRS duration from 105.1±12.0 milliseconds at baseline to 96.8±8.8 milliseconds (95% confidence interval for the change in QRS duration, −4.5 to −12.1 milliseconds). Epinephrine infusion significantly prolonged the QRS duration to 110.5±11.8 milliseconds (95% confidence interval for the change in QRS duration, 1.7 to 9.1 milliseconds). Isoproterenol infusion led to a significant shortening of the QRS duration to 99.6±12.6 milliseconds (95% confidence interval for the change in QRS duration, −1.8 to −9.2 milliseconds). The QRS duration during the first postexercise recording was mildly shortened to 102.1±12.8 milliseconds, but this did not reach statistical significance (95% confidence interval for the change in QRS duration, −6.7 to 0.7 milliseconds). There were no significant sequential changes in the QRS duration during the subsequent two recordings (Table 1). The changes in RMS and LAS induced by these conditions are displayed in Table 1.

Sequential and Combined \( \beta \)-Adrenergic and Parasympathetic Blockade

Fig 4 depicts the individual changes in QRS duration noted with \( \beta \)-blockade, double blockade, parasympathetic blockade, and tilt after double blockade. Tilt after double blockade shortened the QRS duration in all subjects (97.5±9.0 milliseconds). There was no significant difference between the degree of shortening observed with tilt in the presence or absence of double blockade. \( \beta \)-Adrenergic blockade had no significant effect on the QRS duration (106.8±10.8 milliseconds).
When atropine was added, the QRS duration shortened to 103.2±10.3 milliseconds (95% confidence interval for the change in QRS duration, −7.4 to 0.2 milliseconds). On day 2, atropine caused a significant shortening of the QRS duration from 107.9±12.8 to 102.9±11.5 milliseconds (95% confidence interval for the change in QRS duration, −8.4 to −1.6 milliseconds). The changes in RMS and LAS induced by these conditions are shown in Table 1.

**α-Adrenergic and Parasympathetic Stimulation**

Administration of phenylephrine leads to direct α-adrenergic stimulation and a baroreflex-mediated increase in parasympathetic tone. During the initial phenylephrine infusion (combined α-adrenergic and parasympathetic stimulation), there was no significant change in QRS duration, RMS, or LAS (Table 1). Phenylephrine infusion after parasympathetic blockade (pure α-adrenergic stimulation) also caused no appreciable change in QRS duration, RMS, or LAS compared with parasympathetic blockade alone (Table 1).

**Plasma Catecholamine Levels**

Baseline epinephrine and norepinephrine levels were 0.60±0.41 and 1.34±0.77 pmol/mL, respectively. With tilt, there was no significant change in the epinephrine level (0.66±0.44 pmol/mL); however, the norepinephrine level increased to 3.56±2.11 pmol/mL. After double blockade, a similar pattern was observed in the plasma catecholamine levels. Baseline epinephrine and norepinephrine levels were 0.74±0.79 and 1.36±1.03 pmol/mL, respectively. With tilt, the epinephrine and norepinephrine levels were 0.93±0.53 and 3.30±2.06 pmol/mL, respectively.

**Reproducibility of Signal-Averaged ECG Parameters**

The intraclass correlation coefficient for each signal-averaged ECG parameter is depicted in Table 2 for the multiple 5-minute recordings during each condition. No intraclass correlation coefficients are reported for the postexercise period because it is a time-varying condition that would not be expected to demonstrate a high degree of reproducibility. The intraclass correlation coefficients for the QRS duration and RMS were excellent, ranging from 0.90 to 1.00 for all conditions. For the LAS, the intraclass correlation coefficients were similarly excellent, except during the initial tilt, when it was mildly reduced at 0.73.

**Discussion**

In this study, we explored the effects of a wide variety of autonomic stimuli on the signal-averaged ECG in a group of healthy subjects. The QRS duration was altered by some of the conditions studied. Upright tilt shortened the QRS duration; of note, during tilt in the presence of double autonomic blockade, a similar shortening in QRS duration was noted. β-Adrenergic stimulation with isoproterenol also led to a significant shortening of the QRS duration. In contrast, epinephrine prolonged the QRS duration. The effects of the postexercise period on the QRS duration were variable. Increased α-adrenergic stimulation with phenylephrine did not appreciably affect the signal-averaged ECG. Although parasympathetic blockade caused a mild decrease in the QRS duration, parasympathetic stimulation did not significantly affect the QRS duration. Generally, changes in the RMS and LAS paralleled those of the QRS duration. Our findings show that the signal-averaged ECG does not measure only "fixed" parameters. Although the magnitude of the changes in the signal-averaged ECG parameters induced in healthy subjects was statistically significant, further work is necessary to investigate whether alterations in autonomic tone in patients with heart disease produce clinically important changes in these parameters.

The signal-averaged ECG has been a useful noninvasive test for risk stratification of patients after a myocardial infarction.24,25 The pathophysiological explanation for its usefulness is the identification of areas of slowed conduction that are a prerequisite for reentrant ventricular arrhythmias. Although the substrate or late potential identified by the signal-averaged ECG has been pre-
sumed to be fixed, several investigators have reported some day-to-day variability in the parameters measured by the signal-averaged ECG. In addition, time-dependent changes have been described after myocardial infarction. These changes have been assumed to be related to cell death in the border zone of the myocardial infarction or resolution of myocardial ischemia. The present study suggests that other factors, such as changes in autonomic tone, may also alter the signal-averaged ECG. In fact, as demonstrated in Fig 2, some of the conditions increased the QRS duration to more than 120 milliseconds, decreased the RMS voltage to less than 20 μV, and increased the LAS to more than 40 milliseconds; these changes may convert a “normal” signal-averaged ECG to “abnormal.” Although these “abnormal” results have no clinical significance in the group of healthy subjects we studied, they demonstrate that the conditions tested may produce changes in the signal-averaged ECG of a sufficient magnitude to alter the interpretation of the test using the rigid criteria that currently exist. In addition, it is possible that the criteria for a “normal” signal-averaged ECG need to be redefined for subjects with significant changes in autonomic tone, such as sympathoexcitation. Further work will be required to assess the effects of autonomic tone and other potential confounding variables on the signal-averaged ECG in patients with heart disease, as there is considerable variability in autonomic tone in this population.

Potential Mechanisms

Upright tilt and exercise are physiological stimuli that increase sympathetic tone. The increase in sympathetic tone during tilt was documented in our subjects by an increase in the plasma norepinephrine levels and no change in plasma epinephrine levels, indicating an increase in β-adrenergic neural activity rather than circulating β-agonist activity. This pattern of change in plasma catecholamine levels is typical for upright tilt. Epinephrine and isoproterenol infusions were evaluated to study the effects of circulating β-agonist activity on the signal-averaged ECG as a variety of disease states are associated with elevations in plasma catecholamine levels. The effect of these four stimuli on the signal-averaged QRS duration varied. Several potential factors can be considered to explain these differences.

Because the relative intensity of β-adrenergic stimulation achieved with each of the four conditions differed, it is possible that the QRS duration may have a bimodal response to β-adrenergic stimulation. Furthermore, differences in the relative amounts of β-adrenergic, α-adrenergic, and parasympathetic stimulation may have altered the results. However, the difference in the effects of epinephrine and isoproterenol on the QRS duration are not likely to be related to the α-adrenergic effects of epinephrine as phenylephrine infusion after atropine had no significant effect on the QRS duration (in fact, the trend was to shorten the QRS duration). Similarly, differences in parasympathetic tone appear unlikely to explain the observed differences as tilt has been associated with parasympathetic withdrawal and isoproterenol has been associated with increased parasympathetic tone; both of these conditions decreased the QRS duration. Furthermore, parasympathetic blockade decreased the QRS duration, but parasympathetic stimulation had no effect on the QRS duration. Finally, the QRS duration prolongation with epinephrine infusion is not likely to be related to the mild hypokalemia associated with epinephrine infusion as even severe hypokalemia in dogs has been shown to have little effect on the QRS duration.

The shortening of the QRS duration with tilt after autonomic blockade provides an additional hypothesis. It suggests that the tilt-induced changes in QRS duration

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<th>TABLE 2. Intraclass Correlation Coefficients for the Signal-Averaged ECG Parameters Obtained During Various Conditions</th>
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At indicates atropine; T/β-blockade/At, tilt after double blockade; PE, phenylephrine; RMS, root-mean-square voltage in terminal 40 milliseconds of QRS; and LAS, low-amplitude (<40 μV) signal duration.
are at least in part positional. As tilt or upright posture has been associated with a decrease in end-diastolic volume\(^{39-41}\) and the QRS complex is completely inscribed before the onset of the systolic ejection period,\(^{42}\) it is possible that the end-diastolic volume may affect the QRS duration. This is supported by a computer simulation study that suggested increased cardiac volume increases the QRS duration\(^{43}\) and by a recent preliminary report that the QRS duration and end-diastolic volume are related in patients after a myocardial infarction.\(^{44}\) Isoproterenol infusion has been reported to decrease the end-diastolic volume,\(^{45}\) whereas epinephrine infusion has been reported to have no significant effect on the end-diastolic volume.\(^{16,46}\) Thus, it is possible that volume differences may explain some of the diversity in effects of \(\beta\)-adrenergic stimulation. Further work will be required to evaluate this hypothesis.

**Reproducibility**

Because our recordings were performed in triplicate, we were able to characterize the reproducibility of our results. To ensure stability of each condition, the intraclass correlation coefficients for the RR intervals were calculated for each condition. As expected, measurements during the recovery phase of exercise were not constant over the 15-minute recording period. The recordings made during tilt, which were begun after 5 minutes, were also changing over the 15-minute recording period. Examination of the intraclass correlation coefficient for the final two recordings demonstrated excellent reproducibility. In contrast, the intraclass correlation coefficient for the three tilt recordings after double blockade demonstrated excellent reproducibility. Thus, a stable autonomic state is not achieved until 5 to 10 minutes after initiation of the tilt. All other conditions tested demonstrated excellent stability. The immediate reproducibility of the signal-averaged ECG parameters was excellent, as has been previously reported.\(^{47,48}\) Day-to-day reproducibility of the baseline recordings was also excellent.

**Study Limitations**

The major limitation of the present study is the absence of patients with late potentials. Although we considered including patients with heart disease in this study, we did not believe that we could ethically include a sufficient sample of such patients who could undergo the entire protocol. Thus, rather than limit the scope of the study, we chose to perform a comprehensive evaluation of the effects of autonomic stimulation and blockade in subjects who could tolerate the entire study. As the data demonstrate that autonomic tone can affect the normal signal-averaged ECG, further studies are required to evaluate the effects of autonomic tone on the signal-averaged ECG in patients with heart disease.

Many conditions were tested in this study over a 2-day period. The order of testing was specifically chosen so that the testing could be completed within a 2-day period with a return to baseline heart rate and blood pressure between conditions. Although it is possible that there was a cumulative effect from the various conditions, the magnitude of such an effect is likely very small as testing for each condition was only begun when the subject’s heart rate and blood pressure returned to baseline values. Day 1 studies typically lasted 5 to 7 hours to allow for this. Furthermore, as expected, none of the parameters recorded during \(\beta\)-adrenergic blockade with subjects in the supine position, which was performed at the end of the day, were significantly different from baseline parameters acquired at the beginning of the day. An additional limitation is that noise levels obtained during the signal-averaged ECG recordings varied among the conditions tested, which may affect the QRS duration determinations.\(^{49}\) However, the magnitude of the alterations in QRS duration observed in the present study were greater than the expected changes attributable to differences in noise levels,\(^{49}\) suggesting that our results do not reflect changes in noise levels with the differing conditions.

**Clinical Implications**

Our data suggest that the signal-averaged ECG does not measure ‘fixed’ parameters as the results were altered by the tested conditions. The signal-averaged ECG has been most extensively evaluated in patients with myocardial infarctions. In this population, the finding of a ‘late potential’ has been presumed to be fixed. Similarly, the prognostic value of the signal-averaged ECG in patients with dilated cardiomyopathy\(^{5}\) has been presumed to be related to the detection of some fixed substrate. Further studies are necessary in these patient populations to evaluate the effects of autonomic tone (and perhaps other factors) on the signal-averaged ECG and to define how these factors may influence the prognostic usefulness of this test.

**Acknowledgments**

This study was supported in part by grant RR-00048 from the National Center for Research Resources, National Institutes of Health, and Cardiac Pacemakers, Inc, Minneapolis, Minn. We appreciate the assistance of the staff at the Clinical Research Center and the technical input of Aziz El Idrisi of Corazonix Corporation, whose support was invaluable in completing this project. We thank Dr James Young for analysis of the plasma catecholamine levels.

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Assessment of effects of autonomic stimulation and blockade on the signal-averaged electrocardiogram.

J J Goldberger, M W Ahmed, M A Parker and A H Kadish

_Circulation._ 1994;89:1656-1664
doi: 10.1161/01.CIR.89.4.1656

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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