Comparative Study on the Proarrhythmic Effects of Some Antiarrhythmic Agents

S. Dhein, MD; A. Müller, PhD; R. Gerwin, MSc; and W. Klaus, MD

Background. A main side effect of antiarrhythmic drug therapy is the tendency of these drugs to promote arrhythmia within the therapeutic concentration range, i.e., the proarrhythmic activity of these drugs. However, a model for in vitro assessment, quantification, and comparison of proarrhythmic drug activities was still lacking, and only sparse data were available.

Methods and Results. To analyze the arrhythmogenic risk of common antiarrhythmic drugs in a quantitative and comparative manner, isolated perfused rabbit hearts were treated with increasing concentrations of antiarrhythmic drugs corresponding to low, medium, and high therapeutic concentrations. For analysis of the epicardial activation process, an epicardial mapping (256 unipolar leads) was performed. For each electrode, the activation time was determined. From these data, the origins of epicardial activation ("breakthrough points" [BTP]) were determined. At each electrode, an activation vector (VEC) was calculated giving direction and velocity of the local excitation wave. The beat similarity of various heartbeat (under treatment) compared with control was evaluated by determination of the percentage of identical BTPs (deviation ≤1 mm) and of similar VECs (deviation ≤5°). BTP and VEC were reduced by all antiarrhythmic agents tested (propafenone>flecainide>quinidine>ajmaline>procaniamide>disopyramide>mexiletine>lidocaine>sotalol), indicating a more or less pronounced disturbance of the epicardial activation process. Treatment with propafenone, quinidine, and disopyramide and to a lesser extent sotalol prolonged the activation–recovery interval (ARI). ARI dispersion was greatly enhanced by flecainide and was reduced by sotalol. In addition, it could be shown that propranolol is able to reduce the proarrhythmic action of flecainide. This effect seemed to be due to a reduction of the flecainide-induced increase in ARI dispersion.

Conclusions. From the results of our study, we propose the following rank order of the arrhythmogenic risk: flecainide>propafenone>quinidine>ajmaline>disopyramide>procaniamide>mexiletine>lidocaine>sotalol. Moreover, we conclude that propranolol given additionally may be helpful in reducing the proarrhythmic risk of flecainide. (Circulation 1993;87:617–630)

KEY WORDS • proarrhythmia • antiarrhythmic drugs • arrhythmia • epicardial mapping

It has long been recognized that antiarrhythmic drugs may promote arrhythmia.1–3 It could be demonstrated that some cases in which patients suddenly died during antiarrhythmic drug therapy were attributable to the antiarrhythmic therapy.4 Such arrhythmogenic effects occur with therapeutic drug concentrations and therefore do not seem to be a manifestation of toxicity5 but may be a consequence of the electrophysiological alterations induced by the treatment.

Recently, the problem of proarrhythmic drug action has become evident in the course of the Cardiac Arrhythmia Suppression Trial (CAST).6 Unexpectedly, an excess of deaths was observed in a group of patients receiving flecainide or encainide in the postinfarction period.6 But conclusive hypotheses on the underlying mechanism are still lacking. Since the first publication of preliminary results of this study,7 the real arrhythmogenic risk of the antiarrhythmic drugs remains quite uncertain. The definition for proarrhythmic effects used in previous studies was an increase in the frequency of ventricular premature beats or an aggravation of arrhythmia according to the Lown classification.6,8–10 However, during the whole course of the CAST, flecainide and encainide did not exert any proarrhythmic effects according to these definitions. Nevertheless, an excess mortality (due to arrhythmia or to shock after acute recurrent myocardial infarction) during treatment with flecainide or encainide was observed. That means that the proarrhythmic risk cannot be measured only by these conventional parameters. Therefore, we tried to find out 1) whether other changes are induced by these drugs that might be considered arrhythmogenic and might be predictive for the later precipitation of arrhythmias and 2) whether antiarrhythmic drugs in therapeutic concentrations may lead to changes in the cardiac excitation process that later may provoke arrhythmia or can be considered as being proarrhythmic.

In the past few years, we developed an experimental model that allows the assessment of arrhythmogenic drug activity.11–15 The basis of this method is the mapping of the epicardial excitation process in the isolated heart. We demonstrated in a recent study that several kinds of arrhythmia were preceded by marked alter-
ations in the geometry of the activation process before any change in periodicity could be detected:13,15 therefore, we decided to evaluate possible arrhythmogenic drug activity by examination of the influence of these substances on the geometry and time course of the cardiac excitation process. In the study mentioned above,13 a progressive concentration-dependent alteration of the primary epicardial excitation pattern (reduction of beat similarity between different heartbeats) could be demonstrated when arrhythmogenic stimuli were applied. Finally, this reduction of beat similarity of the activation pattern was followed by arrhythmias that were characterized by total loss of similarity (compared with control conditions).

Since the goal of the present study was to investigate possible indicators for the proarrhythmic risk of a prophylactic treatment with antiarrhythmic drugs in a quantitative and comparative manner, the parameters were assessed during the application of the antiarrhythmic drugs at the physiological heart rate (i.e., supraventricular pacing at 180 beats per minute).

In this study, we present the results for several commonly used antiarrhythmic drugs in therapeutic concentrations. Moreover, possible mechanisms of proarrhythmic drug activities presumably leading to dyssrhythmia are discussed. In addition, we present data demonstrating a reduction of flecainide-induced proarrhythmic activity by propranolol.

Methods

Experimental Setup

A computer-assisted 256-channel mapping system (HAL3, developed in cooperation with ELSA GmbH, Aachen)11 was used for simultaneous recording of up to 256 unipolar epicardial leads from isolated rabbit hearts. The circuit resembled a unipolar measurement with electrical ground for reference. The system and the methods are described in detail in previous publications.11-13,15 Briefly, 256 AgCl electrodes were cast in four polyester plates (in 8x8 orthogonal matrices) with 1-mm inter electrode distance. These plates were attached smoothly (in an elastic manner, see Dhein et al12,13) to the heart's surface (without altering coronary flow or left ventricular pressure and without any deflection of the ST segment of the epicardial ECG) so that they could easily follow the heart's movements. The plates were not dislocated in the course of the experiments, as could be verified by the observation of an unchanged shape of the QRS complex in the epicardial potentials (eight of which were observed on-line). The electrodes were connected to the inputs of the amplifier/multiplexer analog/digital converting system HAL3 for recording. Data were stored in 1-MB RAM and then optionally transferred to a common PC system. The temporal resolution was 4 kHz per channel at a bandwidth from 0.15 Hz to 5 kHz (channel coupling < -60 dB), spatial resolution was 1 mm, and voltage resolution was 0.04 mV.

Experimental Procedure

Experiments were performed according to the rules for good laboratory practice and in accordance with the ethical rules of the Council for International Organization of Medical Science and with the German laws for animal welfare.

Male New Zealand White rabbits (commercially, normally fed, 1,600–2,000 g; Fortkamp, Lengerich, FRG) were treated with heparin (1,000 units/kg i.v.) 5 minutes before they were killed by a sharp blow on the neck and exsanguinated. The heart was excised and prepared according to the Langendorff16 technique (constant perfusion pressure, 70 cm H2O; perfusion with Tyrode's solution at 37°C, equilibrated with 95% O2 and 5% CO2; composition of Tyrode's solution [in mmol/l]: Na+ 161.02, K+ 5.36, Ca2+ 1.8, Mg2+ 1.05, Cl− 147.86, HCO3− 23.8, PO42− 0.42, and glucose 11.1). The hearts were paced at a constant frequency (3 Hz) with rectangular pulses of double threshold strength via two right atrial platinum electrodes. After 1 hour of equilibration under standard conditions, the antiarrhythmic agents were added to the perfusion solution in increasing concentrations that corresponded to low, medium, and high therapeutic concentrations (considering the plasma protein binding, Table 1).

For this study, only heartbeats under rhythmic conditions were evaluated. All parameters were assessed

| Table 1. Concentrations of Antiarrhythmic Agents Corresponding to Low, Medium, or High Therapeutic Concentration With Regard to Plasma Protein Binding of Drugs |
|---------------------------------------------|---|---|---|
| Drug                      | A | B | C | Source of drug |
| Quinidine                 | 1 | 5 | 8 | Sigma, St. Louis, Mo. |
| Ajmaline*                 | 0.08 | 0.4 | 2 | Giulini, Hannover, FRG |
| Procainamide              | 20 | 40 | 60 | Sigma |
| Disopyramide              | 1 | 2 | 4 | Searle, Dreieich, FRG |
| Lidocaine                 | 2 | 5 | 10 | Sigma |
| Mexiletine*               | 1.5 | 4 | 6 | Boehringer, Ingelheim, FRG |
| Propafenone*              | 0.08 | 0.4 | 0.9 | Knoll, Ludwigshafen, FRG |
| Flecainide*               | 0.1 | 0.5 | 1.5 | Kettelhack-Riker, Borken, FRG |
| Propranolol*              | 0.01† | 3.0 | 7.0 | Rhein Pharma, Finkenstein, FRG |
| Sotalol                   | 0.8 | 3.0 | 7.0 | Bristol, Troisdorf, FRG |

A. low concentration; B. medium concentration; C. high concentration.
*Standard injection solution; all drugs were dissolved daily in aqua destillata as stock solutions of either 1.0 or 0.1 mmol/l.
†Used only in combination with flecainide; see text.
during periods of constant cycle length pacing of at least 4 minutes. If sustained arrhythmias occurred, the experiments were excluded from further analysis and evaluated separately.

As a first step in data analysis, the activation time points at each electrode were determined as the time point of the most rapid negative intrinsic deflection according to Durrer and Van der Tweel. The coincidence of the fast upstroke of intracellular recorded action potentials with the most negative deflection of simultaneously recorded unipolar epicardial potentials was later also demonstrated by Spach and Dolber and by Smeets and coworkers. The repolarization time was assessed as the time point of maximum dV/dt during the T wave in accordance with Millar and colleagues. The difference between activation and repolarization time points gave the activation-recovery interval (ARI).

ARI was assessed as the mean value of all 256 leads, or alternatively of 64 leads if data for only one region are given. In addition, the homogeneity of ARI was analyzed by determining the standard deviation of ARI in each plate (or heart region: front, left, right, or back wall) as dispersion of ARI.

From the activation time points, it was possible to determine an activation sequence, thereby characterizing the excitation pattern. However, for a more comparative and quantitative analysis, it was necessary to convert this activation sequence (which allows a prime...
**FIGURE 2.** Graphs showing effects of the antiarrhythmic drugs on the relative coronary flow (rCF) in percent of the control value. Absolute control values are given in Table 3. Concentrations (CON) (A, B, C) are given in Table 1. Values are mean±SEM. SEM is indicated by vertical bars if it exceeds symbol size. *, Significant changes. In the lower panel, all changes were significant.

"breakthrough points" (BTPs). These BTPs can be considered the origins of epicardial activation. They were determined for heartbeats under control conditions and for heartbeats under drug treatment. Heartbeats under increasing concentrations of antiarrhythmic agents were then compared with those under control conditions by calculating the percentage of BTPs with locations identical to their location under control conditions (identical defined as deviating not more than 1 mm from their location under control conditions). That means that two identical heartbeats should reveal a BTP similarity of 100%. It is known from previous studies, however, that identical heartbeats occur only rarely and that proarhythmic stimuli reduce BTP similarity. From the data obtained in the above-mentioned studies, we were able to define a lower limit below which it was only a matter of time until arrhythmia would occur; this limit was 50%.

The spread of epicardial excitation was analyzed in a similar way. To allow a quantitative and comparative description of the activation process (as primarily characterized by the activation sequence), for each electrode

an activation vector (VEC) was calculated from the activation times and the locations of the surrounding electrodes, which were activated after the central electrode (i.e., a maximum number of eight), as described by
Müller et al. These VECs give direction and apparent velocity of local activation. The percentage of similar VECs between heartbeats under increasing drug concentrations compared with those under control conditions was determined (VECs deviating not more than 5° from their original direction were considered to be similar). The critical value (see above) for VEC similarity is 10%.

Taken together, the parameters BTP and VEC characterize the activation pattern and sequence, i.e., the geometry of the epicardial activation process, and represent the beat similarity of the cardiac impulse compared with heartbeats under control conditions. Thus, decreasing values for BTP or VEC indicate progressive deviation from the initial (control) activation pattern.

The dynamics of the epicardial activation process were analyzed by four additional parameters.

For a first approach, we determined the total activation time (TAT) in every heart region as the latency between the activation time points of the first and the last electrode being activated in a given heart region. This parameter contains information on the overall epicardial excitation process (with the myocardium as well as the specific conduction system being involved).

For a more detailed analysis, the unipolar potential recordings were evaluated. We determined the latency between the stimulus and the ventricular R spike as PQ time indicating the atrioventricular conduction time. Moreover, the duration of the QRS complex of the unipolar epicardial action potentials was evaluated as an additional velocity-dependent parameter.

Moreover, the epicardial apparent velocities between the electrodes (i.e., the length of each activation vector) were calculated as the mean apparent velocity in each region (VEL). This parameter is different from the real epicardial activation velocity because it measures the latency of activation between two distinct points. That means that this parameter is an integral over all velocities between those points and is influenced by changes in the activation sequence. In this way, the parameter gives an impression of the overall dynamics of the excitation process.

To assess the stability of the electrophysiological parameters, several control experiments were performed in which the time course of changes in the parameters was determined. BTP and VEC similarity remained stable for at least 75 minutes. The values obtained in the control experiments for two heartbeats 30 minutes apart were used as control values in this study (i.e., the control values shown in Figures 4, 5, and 8). In addition, no significant differences in any of the other parameters were observed between the values obtained for the control measurement and values obtained 60 minutes later.

Moreover, the common functional parameters coronary flow (CF) and left ventricular pressure (LVP) were measured continuously. LVP was assessed by insertion of a balloon catheter, which was connected to a pressure transducer (Statham, Fa. Hugo Sachs Elektronik, Hugstetten, FRG) and a bridge amplifier (2-Kanal-Brück enverstärker, Fa. Hugo Sachs Elektronik) in the left ventricle (via the left atrium). CF was determined by direct volumetric measurements. We calculated the relative CF (rCF) as CF/(LVPxHR) (HR, heart rate). A survey of the electrophysiological parameters is given in Table 2.

All drugs were used in three concentrations that were applied in a cumulative manner. These concentrations correspond to a low [A], medium [B], or high [C] therapeutic concentration. These concentrations were chosen with regard to the plasma protein binding according to data from the literature.

All values are given as mean±SEM of six or more experiments. Statistical significance was analyzed by a rank test (Mann-Whitney U test or Wilcoxon test) at a level of 0.05. In the following text, all alterations mentioned were significant compared with control values if not stated otherwise. Concentrations are indicated in brackets ([A], low concentration; [B], medium concentration; or [C], high concentration).

Results

Because in the following text, parameter changes are expressed primarily as percentages, absolute control values are provided in Table 3 as a reference.

All drugs caused a concentration-dependent decrease in systolic LVP (in a range between −10% and −30% in the lowest concentration and between −30% and −50% in the highest concentration, Figure 1). The strongest effects on LVP ensued with propafenone and disopyramide. In parallel to the falling LVP, CF was decreased. However, the ratio between LVP and CF given by rCF was slightly increased except under the influence of propafenone, which enhanced rCF markedly up to 180% (Figure 2).

The mean ARI (ARI of all 256 leads) was increased by most drugs tested (high concentration, [C]): quinidine (+35%) > disopyramide (+24%) > propafenone (+21.5%) > ajmaline (+14%) = sotalol (+14%) = flecainide (+13%) > procainamide (+10%) > lidocaine (+9%) > mexiletine (0%) (Figure 3 and Table 4). These changes were homogeneously distributed over the heart’s surface except under the influence of procainamide and propafenone. These two drugs affected mainly left ventricular ARI (increase by 13%, p<0.05, or 28%, p<0.05, respectively, high concentration [C]) but had only little effect on the ARI in right ventricular leads (increase by 2%, NS, or 13%, p<0.05, respectively, high concentration [C]). The mean ARIs of all heart regions are shown in Figure 3 for all drug concentrations. Absolute values are given in Table 4.

The standard deviation of ARI (i.e., ARI dispersion) was increased only by flecainide. This drug increased ARI dispersion from initially 9.3±0.9 msec by +2±8% ([A], NS, +30±11% [B], p<0.05), and +42±14% ([C], p<0.05; mean values of 256 leads) in a concentration-dependent manner (see Figure 8). In contrast,
sotalol significantly decreased ARI dispersion in a concentration-dependent manner (−13% [A,B], p <0.05; −17% [C], p <0.05). It was the only drug (of all drugs examined up to this point) that led to a significant reduction in ARI dispersion, so this unique effect seems to be remarkable. ARI dispersion was also decreased by propranolol (0.01 µmol/l), but this effect was only a tendency and not significant (reduction by 4.8% [A], 12.4% [B], and 14% [C] from initially 13.5±1.1 msec). The other drugs had no effect on ARI dispersion.

The hearts were found to be activated by a total of 15–25 BTPs. All substances diminished the percentage of identical BTPs compared with heartbeats under control conditions in a concentration-dependent manner (Figure 4). Propafenone had a significant effect in all concentrations and decreased BTP similarity to critical values <50%, whereas flecainide reduced BTP similarity only in the medium and high concentrations down to values near the critical range (50%). All the other drugs exhibited significant effects on BTP only in the high concentrations but never reduced BTP similarity to critical values. We found the following rank order (the percentage of identical BTP under drug treatment [high concentration] compared with control conditions is given in parentheses; a low value, therefore, indicates a high degree of alteration of BTP pattern): propafenone (40% identical BTP)>flecainide (50% identical BTP)>disopyramide (57% identical BTP)>quinidine =procainamide =ajmaline =sotalol (=60% identical BTP)>mexiletine=lidocaine (=65% identical BTP) (see Figure 4).

A variation of BTP means a change in the fixed points of epicardial excitation, which as a consequence should result in an alteration of the propagation directions of the excitation waves. Therefore, the pattern similarity as described by the VEC was found to be decreased by the drugs in a similar way but with slightly changed rank order (Figure 5). Propafenone again exhibited the strongest action beginning with the lowest concentration, resulting in a depression of VEC similarity down to critical values <10%. Similarly, VEC was reduced by quinidine to comparable values starting at the middle concentration. Disopyramide showed a marked depressant effect on VEC in all concentrations ([A] ≈20%, [B] ≈15%, and [C] ≈10% VEC similarity). Ajmaline had no effect in the low therapeutic concentration, then a moderate action [B], but finally led to a strong decline in VEC. Application of the other drugs resulted in a concentration-dependent linear decay of pattern similarity (VEC). Quinidine, disopyramide, ajmaline, and flecainide did not differ in the end points (11%, [C]) of their effects. Sotalol exhibited only moderate effects on this parameter (18% VEC similarity, [C]).

Beginning with maximum effect, the rank order was propafenone >flecainide =quinidine =disopyramide =ajmaline =mexiletine =procainamide =lidocaine =sotalol. The effect of ajmaline on VEC was very moderate in the low and medium concentrations [A, B]. Only application of the highest concentration [C] resulted in a marked aggravation of activation pattern derangement (Figure 5). That means that the epicardial activation pattern as described by BTP and VEC was maximally disturbed by propafenone and flecainide followed by quinidine and ajmaline but was only slightly affected by mexiletine and lidocaine.

For evaluation of the dynamics of the epicardial activation process, we used the parameters TAT, QRS, and VEL, which, to different degrees, depend on specific conduction system and myocardial conduction, respectively.

The TAT was prolonged by most drugs. Whereas a steady and moderate increase was observed with most drugs, the increase caused by propafenone and flecainide was considerably larger, and a sudden strong increase in the effect was seen after the applied drug concentration was raised from medium to high (Figure 6, Table 4). Lidocaine and mexiletine did not cause noteworthy alternations of TAT. The rank order of the drugs was (concentration [C]): propafenone (+180%)>quinidine=flecainide (+75%)>ajmaline (+60%)>disopyramide (+50%)>procainamide (+30%)>lidocaine=mexiletine (+8%)>sotalol (NS) (Figure 6, Table 4).

In contrast to TAT, the apparent epicardial velocities were diminished steadily in a concentration-dependent manner by nearly all drugs (Figure 7). Propafenone diminished VEL more pronouncedly than the other agents. The class 1a drugs and flecainide attenuated the VELs to a similar moderate extent, whereas lidocaine and mexiletine had only minor effects. The apparent epicardial activation velocities were decreased in the

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<th>Table 4. Absolute Drug-Induced Changes in Activation-Recovery Interval and Total Activation Time</th>
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<td><strong>Total activation time (msec)</strong></td>
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Concentrations A, B, and C are given in Table 1. Values are mean±SEM. *Significant changes.
following rank order (concentration [C]): propafenone (-42%) > flecainide > ajmaline (-28%) > quinidine = disopyramide = procainamide (= -20%) > mexiletine = lidocaine (-15%) > sotalol (NS) (Figure 7).

The QRS complex was prolonged by most drugs. Maximum effects were obtained with propafenone (+180%), followed by flecainide and quinidine (+60%) (see Figure 3B). In general, these data were very close to those for TAT (except for propafenone). Therefore, they are not described in detail and are summarized in Table 5. In contrast to the sudden increase in TAT observed with propafenone in the highest concentration, the QRS duration was increased steadily.

In addition, the atrioventricular conduction time was determined by measurement of the PQ interval. We found that the PQ interval (normal range between 60 and 65 msec) was not altered by lidocaine and mexiletine. A slight atrioventricular delay (about 10%) was observed with procainamide and sotalol, whereas disopyramide, quinidine, ajmaline, and flecainide increased PQ interval markedly by up to 40–50%. Propafenone caused the most pronounced atrioventricular retardation by prolonging PQ interval by 85±5%. For both class Ic drugs (flecainide and propafenone), a distinct increment in the effect was detected after the drug concentration was raised from medium to high.

To complete the analysis of the epicardial potentials, we determined the deviation of the ST segment of the unipolar electrograms from the isoelectric line. For this purpose, all positive and, separately, all negative deviations (from zero potential) of all electrograms at the time point 50% local ARI were summed up. Under control conditions, there is a slight elevation of ST segment. Only sotalol significantly altered this parameter (reduction from initially 51±14 mV for 256 electrodes to 63.1±7.4%, [A]; 64.3±8.7%, [B]; and 57.0±8.3%, [C], p<0.05).

Moreover, we evaluated the incidence of arrhythmias. Only during perfusion with the high concentration of flecainide did sustained arrhythmias occur in some cases (three of 12 experiments) when application of this
FIGURE 6. Graphs showing effects of the antiarrhythmic drugs on the total activation time in percent of the control value. Absolute control values are given in Table 3. Concentrations (CON) (A, B, C) are given in Table 1. Values are mean±SEM. SEM is indicated by vertical bars if it exceeds symbol size. *, Significant changes.

concentration lasted longer than 20 minutes. These arrhythmias were sustained ventricular tachyarrhythmias with varying points of origin, thus reminiscent of torsade de pointes arrhythmia. Under the influence of the other drugs, only some extrasystoles but no trains of sustained arrhythmia could be observed. As pointed out above, the different antiarrhythmic compounds did not differ only gradually but rather exhibited clearly different profiles of action (Table 6).

Because of the results of Myerburg et al,25 who showed decreased proarrhythmic action of flecainide in the presence of propranolol (=98–150 μg/l total plasma concentration, corresponding to free plasma concentrations =0.01–0.05 μmol/l), we decided to investigate this drug combination in our model. Therefore, flecainide ([A] 0.1, [B] 0.5, [C] 1.5 μmol/l) was applied in the presence of propranolol (0.01 μmol/l). As can be seen from Figure 8, the reduction of VEC similarity and BTP similarity by flecainide was found to be attenuated. Although flecainide itself induced heterogeneity of ARI and caused a concentration-dependent increase in ARI dispersion (up to +42±14%, high concentration), this effect was no longer seen during combined treatment with flecainide and propranolol. With combined treatment with propranolol and flecainide, we observed no significant increase in ARI dispersion ([A] –8.5±4.9%, [B] +8±7%, [C] +18±11.5%, p<0.05 versus treatment with flecainide alone). Thus, addition of propranolol led to a significant reduction in ARI dispersion compared with flecainide treatment alone. ARI prolongation by flecainide (around 13% if applied alone, high concentration) was only slightly accentuated by the additional treatment with propranolol (+17.4±1.5%, [C]). Under combined treatment with propranolol and flecainide, arrhythmias did not occur.

Discussion

Methodological Considerations

For an exact comparative analysis of the epicardial excitation patterns, a constant location of the measuring plates (electrodes) is necessary. We could verify that the electrodes were not dislocated during the experimental procedure, because in on-line displayed epicardial potentials the QRS complex remained unchanged, whereas any dislocation of an electrode led to a sudden
marked change in QRS morphology. The stability of QRS morphology was controlled during the whole course of the experiment. Moreover, physiological epicardial conduction blocks resulting from large epicardial vessels were observed with constant location during the whole experimental procedure.

The attachment of the plates did not provoke any change in CF, LVP, rCF, or end-diastolic pressure, nor could we register any deviation of the ST segment from zero potential. Thus, the attachment of the electrodes did not injure the hearts. In contrast, methods using electrode arrays sutured to the heart’s surface may provoke injury to the hearts by piercing.

In previous studies, we were able to demonstrate that under arrhythmogenic conditions (e.g., hypokalemia or ouabain treatment), BTP and VEC similarities were decreased in a concentration-dependent manner until finally arrhythmia occurred. This process is characterized by a progressive derangement of the epicardial activation pattern. This situation of altered epicardial excitation that precedes arrhythmia was defined as prearrhythmia. Thus, loss of BTP or VEC similarity indicates alteration of the primary epicardial excitation pattern and is considered to represent an equivalent to proarrhythmic activity.

We decided to use BTP or VEC similarity as a more indirect approach for comparison of the epicardial activation pattern because, unlike isochron representation (or activation sequences), it allows a quantitative assessment of changes induced by drug treatment. This approach has the advantage that it allows comparison of different drugs even if they are not tested in the same preparation and, thus, seems to be superior to other methods representing the cardiac excitation process, at least in comparison of drug effects.

Previous experiments showed that under control conditions, 70–80% of the BTP remained constant and VEC similarity ranged only from 25% to 35%. Breakthrough of epicardial activation is considered to be caused either by underlying Purkinje fibers or by more or less accidental breakthrough of intramurally propagating activation waves. The BTP with constant location (=70–80% of the BTP) are presumably predefined anatomically by Purkinje fibers, whereas the variable BTP (20–30% of the BTP) is considered to result from breakthrough of intramural waves. Loss of BTP similarity, therefore, means an action on the conduction system rather than on myocardial propagation of the activation wave front.

As a consequence of the variability of BTP (20–30%), the excitation pattern as described by the vector fields exhibited a greater range of variation so that only 25–35% of the vectors were classified as being similar. In addition, this variability may also be caused in part by the nonuniform anisotropic properties of cardiac tissue. Hence, vector field similarity is more dependent

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<th>TABLE 5. Effects of Antiarrhythmic Drugs on QRS Duration</th>
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*Significant changes.

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<th>TABLE 6. Synopsis of Changes of Epicardial Activation Process Induced by Antiarrhythmic Compounds</th>
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LVP, left ventricular pressure; BTP, breakthrough point; Vec, vector similarity; Vel, mean apparent velocity; ARI, activation–recovery interval; Disp, ARI dispersion.
–, Decrease; +, increase; (+), moderate effect; 0, no effect. For more details, see text.
on myocardial conduction than BTP similarity. We therefore assume that loss in VEC similarity reflects action mainly on the myocardial conduction rather than on the specific conducting system.

Considerations on data weighting and computational algorithms (concerning calculation of activation vectors) are discussed in detail in a previous publication.15

**Drug Concentrations**

The aim of this study was to evaluate possible proarrhythmic effects of antiarrhythmic drugs. Since it is important to know whether this side effect occurs even in therapeutic concentrations or is a manifestation of toxicity, we investigated three concentrations of each drug. These concentrations were chosen to reflect 1) a low therapeutic concentration [A] with only small effects; 2) a common, medium therapeutic concentration [B]; and 3) a high therapeutic or subtoxic concentration [C]. To achieve this goal, we referred primarily to data from the literature23–25 and calculated the desired concentrations taking into account plasma protein binding. However, since an animal model was used in this study, we had to check whether these calculated concentrations satisfied the above-mentioned conditions in the animal model also. Hence, we tested the respective concentrations as well as one lower and one higher concentration in preliminary experiments. We always found the first dose to be ineffective and the largest dose to be toxic (sustained arrhythmias, atrioventricular block II or higher, reduction in LVP >50%, elevation of end-diastolic pressure). Thus, the medium three concentrations that were finally included in the study (see Table 1) covered the therapeutic range in the animal model, too. This choice allowed a comparison of the arrhythmogenic risk of the drugs in the normal therapeutic concentration range.

In high concentrations, some of the drugs examined in this study exhibited a strong reduction in LVP (30–50%), but these effects were not accompanied by an elevation of end-diastolic pressure or electrophysiological alterations indicating toxicity. Since it is well known that in isolated heart preparations, negative inotropic drug effects are more pronounced than in in vivo models and since the other parameters did not show comparable alterations, this effect might be specific for the model used. Hence, we decided to include these concentrations in spite of this effect.

**Assessment of Proarrhythmic Drug Activity**

Proarrhythmic drug activity has been defined as increased occurrence of arrhythmias or an aggravation of arrhythmia according to the Lown classification.9,10 The CAST, however, demonstrated the failure of this definition, for the mortality was higher in the treated group even though no proarrhythmic effects according to the above definition were seen. Hence, we decided to look for other indicators of proarrhythmic drug activity. In earlier studies from our laboratory, we could show that arrhythmogenic stimuli (e.g., hypokalemia, ouabain intoxication) finally led to the expected arrhythmia, which, to our surprise, was preceded by a period of regular rhythm but altered epicardial excitation spreading. Therefore, analysis of rhythm shows only a sudden onset of arrhythmia, whereas epicardial mapping can reveal changes in the geometry of the excitation process at times when rhythm is still undisturbed. These changes were dose-dependent and finally resulted in VEC and BTP similarities of <10% and <50%, respectively. In addition, it could be shown that it was only a matter of time until arrhythmia occurred, if VEC and BTP similarities were reduced below the critical values mentioned above. The epicardial excitation pattern during
Arrhythmia exhibited no more similarities to the normal (control) spread of excitation, whereas comparison of dysrhythmic heartbeats among themselves showed a considerable degree of similarity. Hence, a progressive, dose-dependent decline in BTP and VEC similarity (compared with control conditions) was assumed to be an indicator of proarrhythmic drug activity in this in vitro model. Therefore, to find out whether there are alterations in the excitation pattern, we decided to compare the spread of excitation in heartbeats during antiarrhythmic drug treatment with that under control conditions. Since these parameters are new, not generally accepted, and not causal but descriptive, other factors generally considered to be causally connected to arrhythmia were included in the analysis of this study. These factors are 1) an increase in ARI dispersion, 27–29 This means increased dispersion of refractoriness, which is generally accepted to be an important promoting factor in arrhythmogenesis. 2) A shortening of refractory period, 30, 31 which is known to increase the probability for a premature depolarization to spread out or propagate because of the premature excitability of the neighboring cells, especially in the presence of critical dispersion. 29 Refractory period was shown to be closely correlated to the epicardial activation–recovery interval in unipolar leads. 20 According to Miller and coworkers, 20 alterations in refractory period in particular are accurately reflected by changes in ARI. Thus, refractory period and especially changes in refractory period can be approximated from measurements of ARI. 3) A decrease in wave length of cardiac impulse (refractory period times conduction velocity), 19, 32 which also may be a promoting factor in the manifestation of a reentry circuit. 4) Extreme prolongation of ARI, possibly leading to a long QT syndrome and arrhythmia as discussed by El-Sherif 33 and Habbab and El-Sherif. 34 These factors may provoke transition from triggered activity or early or delayed afterdepolarization of a single cell to manifest arrhythmia of the whole heart. 29

All drugs caused a derangement of the epicardial excitation pattern, which may lead to arrhythmia, as postulated by Spach and coworkers. 18, 31, 35 Thus, these changes may be considered indicators of proarrhythmic drug activity. In this study, we tried to quantify such alterations with the help of the parameters BTP and VEC similarity. At present, however, there is no statistical link between these parameters and clinical proarrhythmia. On the other hand, changes of the epicardial activation pattern reflect changes in the normal cardiac electrophysiology. In conjunction with the above considerations, the authors assume these parameters to be in vitro indicators of proarrhythmic drug activity at least in this in vitro model.

In the following paragraphs, we will discuss the potential arrhythmogenic risk of the drugs on the basis of the above considerations. However, we want to emphasize at this point that all these considerations apply only to the model used in this study.

According to these reflections, the proarrhythmic risk of sotalol, lidocaine, and mexiletine seems to be rather low, because there were only minor effects on BTP and VEC similarity. This hypothesis is supported by a lack of ARI shortening and by the fact that the apparent excitation velocities (cf. changes in TAT, QRS, VEL) were only slightly affected by these drugs. This probably is because the lidocaine- and mexiletine-induced sodium channel blockade is more pronounced at higher heart rates (time constant of the recovery of the sodium channels is on the order of 0.2–0.5 seconds for class Ib agents, according to References 36 and 37).

By the same arguments, the proarrhythmic risk of procainamide, disopyramide, and ajmaline must be considered to be distinctively greater. This is because procainamide reduced BTP similarity and apparent epicardial activation velocity to a greater extent than the class Ib drugs. In comparison, disopyramide and ajmaline exhibit a greater influence on vector field similarity, which was more pronounced with disopyramide than with ajmaline. However, since an additional dispersion of ARI was seen with ajmaline, its proarrhythmic risk is assumed to be greater than that of disopyramide. Quinidine's influence on pattern similarity (VEC) was a little more pronounced. For this reason, and because of the extreme increase in ARI caused by quinidine, which may provoke a long QT syndrome (possibly leading to arrhythmia), we assume the arrhythmogenic risk of quinidine to be higher than that of the other class Ia substances. This means that, depending on its extent, prolongation of ARI may be either proarrhythmic or antiarrhythmic. A clear limit separating these actions cannot be given at this time.

In this study, the highest arrhythmogenic risk must be ascribed to flecainide and propafenone, for different reasons. Whereas the reasons for propafenone's high arrhythmogenic risk are its extreme depressant effect on VEC and BTP similarity and on apparent epicardial activation velocities as well as a regionally heterogeneous influence on ARI, the high risk of flecainide is a result of the distinct increase in ARI dispersion. Because several studies demonstrate the importance of an increase in dispersion of refractory period for elicitation and maintenance of arrhythmia, 27–29, 31 we considered this factor to be more important than the effects on epicardial excitation spreading. Thus, flecainide is considered to possess the highest arrhythmogenic risk of all drugs investigated in this study. This conclusion is supported by the observation of arrhythmias under the influence of flecainide. The significance of altered excitation geometry and increased ARI dispersion for the proarrhythmic drug action of flecainide is supported by very recent findings of other authors, who reported on inhomogeneities of the activation and repolarization pattern under flecainide treatment during the postinfarction period in the dog. 38

Following this line of argument, changes in activation pattern can be considered to be related to ventricular proarrhythmic drug actions. Since the discussion above has demonstrated considerable differences in the action of the drugs investigated in this study, it is very tempting to compare the drugs and to propose a rank order of the intensity of alterations of the epicardial activation process in this in vitro model: flecainide > propafenone > quinidine > ajmaline > disopyramide > procainamide > mexiletine, lidocaine. This rank order may also reflect the rank order of ventricular proarrhythmic drug activity in this model. Of course, it must be kept in mind that this ranking refers only to the in vitro model and that, at present, the relations between these findings and clinical proarrhythmia are conjectural. But according to our knowledge and experience, this rank order corresponds.
primarily to rank orders of proarrhythmic effects, which can be inferred from various clinical reports on proarrhythmic effects in individual patients.\(^{23,39-42}\)

The aim of this study was to investigate and compare the proarrhythmic risk of different antiarrhythmic drugs in the same experimental model, not to assess their antiarrhythmic potency. Hence, we used normal hearts and not an experimental model for arrhythmia. Since the situation of a prophylactic treatment with antiarrhythmic drugs should be simulated and a quantitative comparison of the various drug effects should be possible, we decided to pace the hearts at their physiological heart rate (the same rates were used in all hearts for control conditions and for each drug tested). It should be kept in mind, however, that most of the drugs used in this study exhibit rate-dependent effects.\(^{26,43,44}\) It can be speculated that, for example, the actions of class 1b antiarrhythmic compounds such as lidocaine or mexiletine on the excitation patterns will be more pronounced at higher heart rates because of the well-known use dependence of these drugs. Therefore, the findings of this study can be attributed only to the normal physiological conditions (i.e., normal heart rate). At higher heart rates, the proarrhythmic effects may be more pronounced (in some cases) and the ranking may be different. However, including data on rate dependence of the parameters under various drugs (in various concentrations) would be beyond the scope of this paper. Future experimental work is needed to deal with that specific point.

In the following paragraphs, the effects of the class III antiarrhythmic compound sotalol will be compared with those of the class I antiarrhythmic drugs.

The sotalol-induced prolongation of ARI is comparable to or even less than that achieved with several class Ia or Ic antiarrhythmic compounds (e.g., quinidine, disopyramide, propafenone). Therefore, the class III properties of sotalol are probably not restricted to a prolongation of potential duration. The main difference from the other drugs that produce prolongation of ARI is that sotalol has no slowing effect on the propagation velocities. Hence, sotalol, in contrast to class I agents, does prolong the wave length of cardiac impulse. It is possible that class III properties can be better defined by prolongation of cardiac wave length. Future work is needed to test this hypothesis. The prolongation of epicardial potential duration by sotalol probably is a result of an inhibitory action on repolarizing potassium currents.\(^{45,46}\) This point of view is supported by our finding that ST segment elevation (at 50% ARI) was suppressed only by sotalol. ST segment elevation is supposed to result from increased efflux of positive charges, mainly of potassium ions.\(^{15}\)

Interestingly, the dispersion of ARI was reduced by both \(\beta\)-adrenergic receptor blocking drugs. This effect was significant for sotalol but could also be seen to a lesser extent with propranolol. ARI dispersion under control conditions is thought to be a result of the physical properties of the cardiac tissue such as nonuniform anisotropy.\(^{47}\) At present, the underlying mechanism of the sotalol effect on ARI dispersion remains unclear. But this reduction of dispersion and the prolongation of cardiac wave length seem to be responsible for the antiarrhythmic action of sotalol.

Nevertheless, even sotalol altered the epicardial excitation pattern to a degree similar to lidocaine or mexiletine. This may be accounted for by prolongation of ARI, thereby prolonging cardiac wave length, which in consequence may lead to altered pathways of excitation.

Since any change of refractory period induces altered cellular coupling, as demonstrated by other authors,\(^{48}\) and any alteration of wave length alters the excitability of the cells depending on the previous beat history,\(^{49,50}\) changes either in ARI or in local activation velocity can be expected to induce changes in the excitation pattern. Because any such change can be assumed to represent a possible proarrhythmic action, sotalol, too, does possess a proarrhythmic risk. Whereas the proarrhythmic risk of sotalol and, presumably, other class III antiarrhythmic agents is a result of a prolongation of the cardiac wavelength, as claimed by others,\(^{51}\) the proarrhythmic risk of class I antiarrhythmic compounds seems to be associated with a shortening of the wave length of the cardiac impulse. Alteration of wave length, whether prolongation or shortening, includes a more or less pronounced proarrhythmic risk. In the case of class I antiarrhythmic compounds, this alteration of cardiac wave length is a shortening caused by reduction of activation velocity,\(^{10}\) whereas it is a prolongation caused by lengthened action potential duration in the case of class III antiarrhythmic drugs. Although both prolongation and shortening of cardiac wave length mean a proarrhythmic risk, prolongation of the cardiac wave length can be assumed to be less arrhythmogenic, because under these conditions, reentry circuits must have a greater diameter and are therefore less probable.\(^{32,52-55}\) But there may be additional mechanisms in the case of sotalol, because changes in cardiac wave length are relevant to reentry arrhythmias but mostly irrelevant to other mechanisms of arrhythmia.

Finally, the interaction between flecainide and propranolol will be discussed. Since we were able to demonstrate that the proarrhythmic action of flecainide goes along with increased ARI dispersion and loss of VEC and BTP similarity, and since Myerburg et al\(^{26}\) showed the proarrhythmic action of flecainide to be reduced by propranolol, we decided to investigate whether this effect can also be seen in vitro and, if so, to elucidate the underlying mechanism. Our results demonstrate that propranolol can diminish the proarrhythmic action of flecainide in vitro, too. This conclusion is supported by several findings: 1) no more arrhythmias could be observed when flecainide was administered together with propranolol, 2) flecainide-induced ARI dispersion was reduced, and 3) flecainide-induced loss of BTP and VEC similarity was alleviated. Taken together, these findings might be explained by an improved intercellular coupling that can be expected to result in a reduction of dispersion of refractoriness.\(^{47}\)

On the other hand, this finding supports the significance of the parameters used in the present study to characterize proarrhythmic drug activity.

It could be demonstrated that all drugs investigated led to more or less pronounced alterations of the epicardial excitation process before disturbing periodicity. Most, but not all, effects of the substances can be explained by their (different) action on ionic channels and kinetics of action. Moreover, some of the drug effects are already known from other investigations but
were never measured simultaneously in only one model. However, since the aim of this study was to compare the proarrhythmic risks of these agents, an extensive discussion about the ionic mechanisms would be beyond the scope of this study.

Conclusions

From our results, we propose the following rank order of proarrhythmic drug action for this in vitro model: flecainide > propafenone > quinidine > ajmaline > disopyramide > procainamide > mexiletine, lidocaine > sotalol. The findings of our study with regard to flecainide coincide with those of the CAST study and may help to explain the underlying mechanism, which we assume to be dispersion of refractory period.

In summary, these results confirm the hypothesis that all antiarrhythmic drugs may promote arrhythmia and suggest that prophylactic treatment should be restricted to selected cases only. Propranolol may be used as an additional drug for treatment with flecainide, because the arrhythmogenic effects of this class Ic compound could be alleviated by propranolol.

The model described in this study may help to assess the proarrhythmic risk of new drugs in an in vitro model and may give insight into the underlying mechanisms.

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