Instability and Triangulation of the Action Potential Predict Serious Proarrhythmia, but Action Potential Duration Prolongation Is Antiarrhythmic

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Background—Prolongation of action potential duration (APD) is considered a major antiarrhythmic mechanism (class III), but paradoxically, it frequently is also proarrhythmic (torsade de pointes).

Methods and Results—The cardiac electrophysiological effects of 702 chemicals (class III or HERG channel block) were studied in 1071 rabbit Langendorff-perfused hearts. Temporal instability of APD, triangulation (duration of phase 3 repolarization), reverse use-dependence, and induction of ectopic beats were measured. Instability, triangulation, and reverse use-dependence were found to be important determinants of proarrhythmia. Agents that lengthened the APD by >50 ms, with induction of instability, triangulation, and reverse use-dependence (n=59), induced proarrhythmia (primarily polymorphic ventricular tachycardia); in their absence (n=19), the same prolongation of APD induced no proarrhythmia but significant antiarrhythmia (P<0.001). Shortening of APD, when accompanied by instability and triangulation, was also markedly proarrhythmic (primarily monomorphic ventricular tachycardia). In experiments in which instability and triangulation were present, proarrhythmia declined with prolongation of APD, but this effect was not large enough to become antiarrhythmic. Only with agents without instability did prolongation of APD become antiarrhythmic. For 20 selected compounds, it was shown that instability of APD and triangulation observed in vitro were strong predictors of in vivo proarrhythmia (torsade de pointes).

Conclusions—Lengthening of APD without instability or triangulation is not proarrhythmic but rather antiarrhythmic. (Circulation. 2001;103:2004-2013.)

Key Words antiarrhythmia agents ■ torsade de pointes ■ arrhythmia ■ drugs ■ action potentials

Prolongation of action potential duration (APD) is considered to be an antiarrhythmic mechanism and the primary mechanism for class III agents. Unfortunately, prolongation of APD is so frequently associated with proarrhythmic activity (early afterdepolarizations [EADs] and torsade de pointes [TdP]) that it is a basis for alarm: whenever a drug lengthens the QT interval, many clinicians as well as regulatory bodies reflexively become concerned. To the best of our knowledge, no conclusive evidence exists that prolongation of APD inevitably must lead to proarithmetic. If one could prove that prolongation of APD always leads to proarrhythmia, then class III antiarrhythmic agents would become a contradiction in terms, and drugs that prolong APD must then a priori be avoided, unless perhaps their benefit is expected to exceed their harm. In contrast, if the APD can be lengthened in a safe fashion, then this could form an effective antiarrhythmic mechanism. Although it may be difficult or impossible to prove that prolongation of APD is always proarrhythmic, demonstration that the APD can be lengthened safely is readily feasible: one only needs to find 1 agent that does it.

After ∼7000 experiments with the SCREENIT system, the technicians at Hondeghem Pharmaceutical Consulting could frequently anticipate which chemicals were dangerous long before EADs or TdP developed: whenever the action potentials in a train were no longer exactly superimposed, proarrhythmia frequently followed; if not at the present concentration, then commonly when the drug concentration was increased 3- to 10-fold. They also noted that when instability of APD was associated with triangulation (prolongation of APD to APD), then EADs and proarrhythmia were certain to follow.

In the present report, we show that in vitro instability of APD is a strong predictor of in vivo proarrhythmia, especially when associated with triangulation. More importantly, we demonstrate that prolongation of the action potential plateau without instability or triangulation is antiarrhythmic, not proarrhythmic.

Methods

In Vivo Experiments
The method of assessing proarrhythmic potential in the anesthetized rabbit has been described in detail elsewhere. Briefly, 149 male

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Tables I and II can be found Online at http://www.circulationaha.org
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Experimental Procedures

For the in vivo experiments, after baseline measurements of 10 minutes, a continuous infusion of methoxamine (70 nmol · kg⁻¹ · min⁻¹) was started. Ten minutes later, the compound under investigation was infused for 30 minutes; 5 minutes later, the dose was increased up to 10-fold. The first dose lengthened the guinea pig monophasic APD by 20% (Table I). When an episode of TdP was initiated, the experiment was terminated. The RR and QTU (first deviation from isoelectric line during PR interval to second peak of QTU) intervals were interactively measured from averaged (≥10 consecutive beats) computer-sampled ECG signals.

For the in vitro experiments, once the heart was mounted on the experimental station, the execution and analysis of the experiment proceeded without any human intervention. The computer stimulated at 1.5 times threshold stimulation current. If automaticity and escape cycle length were >100 ms, threshold stimulation current <300 µA, coronary perfusion >17 mL/min, ectopic rate <8 bpm, and the cardiac activation time <60 ms, then the preparation was stimulated until instability (determined by the best easy systematic method, described below) of the last 20 trains became <10 ms. Preparations that did not achieve these criteria were rejected.

The experiment consisted of brief protocols (executed every minute) and large protocols (10 minutes in control and highest drug).

Brief Protocol

Stimulation current was readjusted (if necessary), and the action potentials of a 10-beat train at 1000 ms and 300 ms were saved, together with a train of 30 action potentials stimulated at a cycle length of 1000 ms.

Large Protocol

Stimulation current was adjusted, and automaticity, escape cycle lengths, conduction times, and APDs for cycle lengths at 2000, 1500, 1000, 750, 500, 300, and 250 ms were determined. Trains of 5 seconds at cycle lengths of 250, 300, 400, and 500 ms were recorded.

The preparation was perfused with drug-free solution while the brief protocol was executed 10 times, followed by the large protocol (control data). The drug infusion was then started, for 10 minutes at each of the 5 concentrations, with the brief protocol executed each minute. If the APD shortened by 40 ms or lengthened by 80 ms, the “effective” concentration continued for 8 minutes, followed by the large protocol (drug data). When a chemical appeared to be interesting or there was uncertainty as to the full development of the drug effect in only a 10-minute perfusion period, an additional experiment was done perfusing an effective drug concentration for 30 to 180 minutes.

Septal and epicardial monophasic action potentials were digitized at 1 kHz (12 bits). For the conduction data, sampling was done at 10 kHz (each channel). Data were analyzed beat by beat during the experiment, and the results were compressed and saved to disk.

Electrophysiological Determinations

APD₀ to APDₚₙ were measured from the midpoint of the upstroke until 10%, 20%, ... 90% repolarization. As APD₀ to APDₚₙ prolongs, the action potential takes on a more triangular shape. Triangulation is defined as the repolarization time from APD₀ to APDₚₙ. Reverse use-dependence was measured as the difference between the APD₀ of the first 10 and the last 20 action potentials of a 30-pulse train.

Any action potential whose upstroke was not within 80 ms after the stimulus was considered an ectopic. Only action potential amplitudes exceeding ≥50% of the average upstroke in a train, however, were considered valid. The number of ectopic beats (ectopics) reported per minute was obtained as the average during the last 3 minutes at any 10-minute drug exposure. This algorithm underestimated the proarrhythmia, for several reasons: closely coupled ectopics with small amplitude were not counted; when ectopics became too frequent (Tdp, VT), the experiment could not be
continued; and sometimes ectopics developed only transiently and did not occur during the last 3 minutes of drug exposure.

**Chemicals**

All compounds were synthesized at AstraZeneca R&D, Mölndal, Sweden. Stock solutions (tartaric acid [in vivo] or dimethylsulfoxide [in vitro]) were prepared daily and diluted with saline. The basal structure of the compounds for the in vitro study comprised varia-
tions on a known APD-prolonging pharmacophore varied by use of a computer-guided parallel synthesis approach. Only compounds known to prolong the APD or to interact with the HERG channel were submitted to the SCREENIT system for study at concentrations of 0.03, 0.1, 0.3, 1, and 3 μmol/L.

**Statistical Analysis**

The 20 compounds used for in vivo validation of the in vitro data (APD instability, triangulation, and reverse use-dependence) were selected from a large database comprising several hundreds of QT-prolonging compounds tested for proarrhythmic effect in the methoxamine-treated anesthetized rabbit.6,9 “Proarrhythmic” compounds were selected from compounds with a TdP incidence >60%. “Less proarrhythmic compounds” were selected from compounds with a TdP incidence ≤5 (Table I).

Comparison between 2 means was done with Student’s t test, and that between multiple means by ANOVA (Scheffé test; significance was set at 95% confidence). Data are presented as mean±SEM unless explicitly stated otherwise.

Instability of APD was tested with a nonparametric test, because it is not possible to assume that APD is normally distributed during proar-
hythmia, when sometimes only a limited number of “normal” action potentials can be obtained. To minimize the bias induced by a few exceptionally long or short APDs, the best easy systematic6 was used to estimate the APD50: basically, steady-state action potentials were sorted according to their APD50 and by linear interpolation, the median, upper 25%, and lower 25% values were computed. An instability index was obtained by computing the difference between the upper and lower quartile estimates in milliseconds. For the experimental trains in each drug concentration, the last 20 action potentials for the final 3 minutes of drug perfusion were used, i.e., 60 action potentials in total.

**Results**

**Description of Instability and Triangulation**

In Figure 2, during control (before time 0), the APD exhibited little beat-to-beat instability (7 ms). This value is typical of control preparations (7.6±0.2 ms; n=1071). Infusion of almokalant (class III agent)9 progressively lengthened the APD but also increased instability: 19 ms by minute 2 and 85 ms between 4 and 5 minutes. This increased instability of APD was not caused by ectopics: these appeared only in minute 6, when the EADs deteriorated in ectopics and TdP. Thus, instability increased >100% 3 minutes before the first EAD and 4 minutes before TdP developed.

For drugs that induce instability, characteristically a small rhythm disturbance markedly enhances instability (in >11 000 experiments, such instability has never been seen in controls). In Figure 3 (presence of drug; top right), the ectopics induced markedly greater instability of APD than in controls (top left). To visualize APD instability, one can create a Poincaré plot [APD of the nth action potential is plotted against the (n-1)th APD].10,11 In such a plot, identical action potentials project to a single point. If the APD lengths or shortens smoothly, then the points cluster closely around the diagonal line. But large deviations between successive action potentials deviate markedly from the diagonal line. For occasional isolated disturbances, the points will make simple triangular patterns. If the changes in successive action potentials induce changes in subsequent action potentials, however, then complex polygons can develop and the patterns can become chaotic.11 In controls (Figure 3, left middle panel), all 300 points of the Poincaré plot cluster closely, but as a destabilizing drug effect developed (middle panel), deviations from the diagonal line developed. By 23 minutes (right panel), successive action potentials described increasingly complex polygons. The fact that the points describe loops around the diagonal line indicates that the system is not exhibiting random noise but rather deterministic deviations.11 The associated train of action potentials is shown in Figure 3 (bottom).

In >5000 experiments in which instability was measured with the SCREENIT system, no agent that lengthens APD and is proarrhythmic has been able to stay close to the diagonal throughout the experiment. Conversely, when poly-
gons having >3 corners induced large deviations from the diagonal, EADs were always observed, and these frequently deteriorated into TdP.

When APD prolongation results primarily from slowing of repolarization during phase 3, then the action potential becomes more triangular: triangulation. Almokalant (Figure 2) did not lengthen APD to APD50 but markedly prolonged APD50 to APD90 (primarily APD70 to APD90). During minute 5 of perfusion, the slowing of repolarization became so pronounced that repolarization stalled and EADs appeared.

**Can In Vitro Instability and Triangulation Predict In Vivo Proarrhythmia?**

To answer this question, compounds with high and low proarrhythmic potential (10 each) were compared (Table I).
The 20 selected agents were submitted for blind assessment of APD instability and triangulation in 1 single experiment in vitro for each chemical. The drugs were ranked according to the least instability plus triangulation: 705, 855, 609, 865, 476, 142, 213, 566, 454, and 635 (the 2 italicized compounds were classified as having high proarrhythmic potential by the in vivo experiments). In this list of the 10 best agents, none of the compounds were able to lengthen the APD while not increasing instability and not inducing triangulation, ie, SCREENIT declared the 10 “best” agents as not really good. After the code was broken, we concluded that SCREENIT could effectively separate high- and low-proarrhythmia compounds. Consequently, it was decided to apply it to a large series of chemicals (n=702).

Average Drug Effects on Instability, Triangulation, and Ectopics as a Function of Changes in APD

In the absence of chemical, at a cycle length of 1000 ms the mean APD$_{50}$ was 232±1.3 ms (n=1071), and over a 3-minute period, instability was 7.6±0.2 ms. At 1 Hz, APD$_{50}$ exhibited no reverse use-dependence (APD$_{50}$ declined by <1 ms). APD$_{50}$ to APD$_{90}$ lasted 91±0.9 ms, and the preparations generated on average 4.8±0.1 ectopic beats/min (fewer result if the normal external potassium of 4 mmol/L is used).

In Figure 4, all the APD changes (drug/concentration) were grouped in bins of 50 ms. For each bin having ≥20 observations, the average was calculated for triangulation, instability, and number of ectopics (n=3968). On average, minimum instability, triangulation, and proarrhythmia occurred for drugs/concentrations that did not alter the APD. When shortening of APD was proarrhythmic, most commonly it resulted in monomorphic ventricular tachycardia. In contrast, when prolongation of APD was proarrhythmic, it most commonly led to EADs and TdP.

Relationship Between APD, Instability, Triangulation, and Proarrhythmia

All changes of instability as a function of changes in APD are plotted in Figure 5 (top). Most of the 4008 experimental points are superimposed into a confluent cloud, but the more interesting outlyering points can easily be recognized: agents can prolong the APD by ~200 ms while actually reducing instability. A few agents actually lengthened APD by up to 400 ms without increasing instability, and 1 chemical prolonged APD >600 ms without inducing much instability.

Similarly, prolongation of APD on average induced triangulation, but prolongation of APD was not mandatorily associated with triangulation. Indeed, some chemicals length-
ened APD up to ~400 ms while actually reducing triangulation (Figure 5, bottom).

Although on average, prolongation of APD appears to be proarrhythmic (Figure 4), it could be that shortened or lengthened APD is proarrhythmic only when combined with instability, triangulation, or reverse use-dependence but could become antiarrhythmic when ≥1 of these are absent. To test this hypothesis, several data subsets were constructed (Table II) on the basis of whether the chemicals shortened APD by $\geq 20$ ms or lengthened the APD a little ($>20$ ms) or a lot ($>50$ ms). For each of these 3 subgroups, further subgroups were constructed on the basis of whether they increased or decreased instability, triangulation, or reverse use-dependent prolongation of APD.

The most striking aspect of this table is that only drug concentrations that do not induce instability are antiarrhythmic, provided that the APD lengthens. Actually, the most marked antiarrhythmic action is obtained when the APD is lengthened by $>50$ ms, if the action potential also does not exhibit instability, is not triangulated, and does not exhibit reverse use-dependence. Conversely, proarrhythmia is most marked when instability is combined with shortening of APD in the presence of triangulation and reverse use-dependence.

In the group in which APD lengthened by $>50$ ms, the antiarrhythmic action in the absence of instability, triangulation, and reverse use-dependence was $-5.4 \pm 2.8$ ectopics/min ($n=19$), whereas in their presence, there was proarrhythmia of $6.7 \pm 0.5$ ectopics/min ($n=481$), and this difference was highly significant ($P<0.001$). Again, the difference could not be due to the difference in APD prolongation, because removing the longest APDs until APD increased by $72 \pm 0.9$ ms in both groups ($n=219$; see row marked by † in Table II) again increased the proarrhythmia.

To better visualize the effect of instability (left segment), triangulation (center segment), and reverse use-dependence (right segment) on proarrhythmia, these subgroups are shown as circles (Figure 6). The segment was colored red if the parameter was increased and green if reduced. Agents that increase instability, triangulation, or reverse use-dependence (red segments) cluster as being more proarrhythmic. Furthermore, the proarrhythmia is most pronounced for the chemicals with which instability, triangulation, and reverse use-dependence concur (red circles). Conversely, agents that did...
Does Instability Predict Proarrhythmia?

Does instability precede proarrhythmia (as was noted by the technicians) or is it only the result of proarrhythmia? To answer this question, a subdatabase was constructed that held all experiments (n=182) with serious proarrhythmia (>10 ectopics/min) and instability (>20 ms), provided that the proarrhythmic concentration had ≥1 lower concentration with <2 ectopics/min. These restrictions are needed because one must have proarrhythmia and instability to be able to test whether one precedes the other, and there must similarly be a lower concentration in which the arrhythmia is not yet present, because otherwise it can no longer be induced. In 78 experiments, the instability increased by >6 ms at a concentration that was 3-fold lower than the proarrhythmic one; in 29, the instability developed at a 10-fold lower concentration; in 13, instability increased already at a 30-fold lower concentration; and in 5 experiments, instability increased even at a 100-fold lower concentration. These numbers underestimate the power of instability to predict proarrhythmia: the total concentration range in the present experiments is only 100-fold. Thus, the latter 5 experiments required that the arrhythmia developed in the highest concentration studied and that the instability became measurable at the lowest concentration studied. Thus, the real incidence of instability preceding proarrhythmia by 2 orders of magnitude is probably 5 times greater. Nevertheless, because in 125 of 182 experiments, instability preceded proarrhythmia, we must conclude that instability can predict proarrhythmia in ≥69% of cases. In the 57 experiments in which the instability occurred at the proarrhythmia concentration, instability usually preceded the proarrhythmia in time (see for example Figure 2). Unfortunately, we did not keep track of this. Although instability nearly always preceded the proarrhythmia, it certainly was not 100% the case. Indeed, there were a few very exceptional instances in which no instability could be detected before the proarrhythmia, not even in the few seconds before proarrhythmia started.

Comparison of a Proarrhythmic and Antiarrhythmic Prolongation of APD

In Figure 7, 2 agents that markedly lengthen APD are compared. One induces instability and triangulation (experiment 10 802), and the other (experiment 11 037) does not. Indeed, in experiment 10 802, APD<br/> is actually shortened, whereas APD<br/> is markedly lengthened (see top and bottom left panels of Figure 7), rendering the action potential more triangular. The Poincaré plot in the middle left panel demonstrates that as the APD prolongs, the successive APDs become increasingly unstable. Actually, as soon as the APD starts to lengthen by ≈100 ms, there are hardly any more points on or close to the diagonal line; APD prolongation develops in a chaotic fashion. Although this experiment was selected, it is not at all untypical for proarrhythmic class III agents: there are certainly >100 examples in this series that are equally chaotic or worse. The instability is also appreciated from the spread of the individual APD<br/> , APD<br/> , and APD<br/> points in the bottom panel: knowing the current APD, it is not possible to predict the next.

In contrast, the prolongation of the action potential in experiment 11 037 is due to a lengthening of the plateau, ie, there is no triangulation. From the right bottom panel, one can appreciate that the APD<br/> , APD<br/> , and APD<br/> remain close together as the APD lengthens. In the Poincaré plot, all APDs cluster along the diagonal line, exhibiting no deviations that are larger than in control. This profile is unfortunately not very common: in this series of 702 chemicals, only 2 could prolong APD >100 ms without precipitating instability or triangulation.

Discussion

The results demonstrate that the generally perceived notion that prolongation of APD or QT is associated with proar-
rhythmia (including EADs and TdP) is on average correct. Indeed, many more agents prolong the APD with instability, triangulation, or reverse use-dependence than without (see Table II).

The results show that proarrhythmia is frequently (>69% of cases) preceded by instability. Although instability appears to be more common as APD is lengthened, however, the 2 are not causally related: instability frequently develops before APD prolongation (eg, Figure 2); there were also numerous instances of increased instability in the presence of shortening of APD; a few agents lengthened APD without instability.

The results show that in this series of chemicals, prolongation was commonly associated with triangulation. Triangulation is not a mandatory consequence of APD prolongation, however: some agents lengthen the APD exclusively by prolongation of the plateau without phase 3 prolongation; rarely, phase 3 repolarization was even shortened. This squaring of the APD is expected for agents that have an effect on a plateau current and not on a phase 3 current: the longer and perhaps more positive plateau activates more If, so that a faster phase 3 repolarization can follow.

The results suggest that reverse use-dependence may promote proarrhythmia. The importance of this parameter is probably badly underestimated in the present study, because we did not evaluate reverse use-dependence at various frequencies. Thus, all we can conclude is that at the cycle length of 1000 ms, reverse use-dependence appears to enhance proarrhythmia.

Most importantly, the results show that agents can prolong APD without instability, triangulation, and reverse use-dependence.
dependence and that these agents are significantly antiarrhythmic. This is important, because otherwise the class III antiarrhythmic principle would be of limited clinical value. Unfortunately, the antiarrhythmic effect of APD prolongation is easily ruined by the presence of instability, triangulation, reverse use-dependence, or especially by their combination. Because (at least in the present series) these are much more frequently present than absent, it is not surprising that prolongation of the QT is perceived as being dangerous. Thus, our general conclusion is that instability, triangulation, and reverse use-dependence are proarrhythmic, whereas prolongation of APD in their absence is antiarrhythmic.

Relative Proarrhythmic Importance of Instability, Triangulation, and Reverse Use-Dependence

The importance of the above parameters for proarrhythmia is probably not uniform, and they probably also act synergistically. Indeed, when an agent exhibits instability plus triangulation plus reverse use-dependence (solid red circles in Figure 6), then proarrhythmia is most marked. Conversely, when an agent exhibits neither instability, triangulation, nor reverse use-dependence (solid green circles), then the agent tends to be the least proarrhythmic or even antiarrhythmic (at least if the APD is prolonged). In general, agents that exhibit instability (left segment red) tend to perform the worst, whereas agents that do not exhibit instability (left segment green) tend to cluster best. This suggests that under the present experimental conditions, the most important parameter relating to proarrhythmia is instability. Similarly, triangulation (center segment) appears to be the second most important parameter, whereas reverse use-dependence (right segment) appears least important (at least as measured here).

Mechanisms

The APD starts with the upstroke (phase 0) and ends with terminal fast repolarization (phase 3). Because phases 0 and 1 (see Figure 1) are usually relatively short, the APD is primarily the sum of phases 2 and 3. Consequently, the APD can be lengthened by prolongation of phase 2, phase 3, or both. The channels carrying the current during phase 2 and phase 3 are substantially different.12 Indeed, during the plateau, the small declining inward currents flow primarily through slowly inactivating sodium channels and L-type calcium channels, whereas the progressively increasing outward currents flow through potassium channels (to a large extent $I_{Ks}$, because $I_{Ks}$ is inactivated primarily at more positive potentials in the plateau). During phase 3 repolarization, open inward channels promptly close by deactivation, but $I_{Ks}$ and $I_{K1}$ potassium channels promptly open by removing rectification, whereas $I_{Ks}$ slowly deactivates. Conversely, drugs can prolong phase 2 by increasing inward currents (sodium/calcium) or by reducing outward currents ($I_{Ks}$). Drugs can slow phase 3 repolarization by closing $I_{Ks}$ or $I_{K1}$ channels. Promiscuous drugs can prolong both phases 2 and 3 by interacting with ion channels of both groups. (The above is a conceptual simplification, because surely there is some minor activity of all these channels during both the plateau and repolarization; also, there are numerous additional channels that are beyond the scope of the present concept; block of $I_{Ks}$ channels may also extend the plateau repolarization, because more repolarization is necessary to recruit enough channels to speed up repolarization).12 Agents acting primarily on 1 type of channel are therefore anticipated to either primarily prolong the plateau or primarily slow phase 3 repolarization. It therefore becomes of interest to evaluate whether prolongation of phase 2 versus phase 3 is more or less proarrhythmic.

Slowing of phase 3 repolarization (triangulation) has $\approx$4 reasons for being potentially dangerous. First, spending too much time in the window voltage for calcium channel reactivation can generate EADs early during repolarization.14 Second, remaining too long in the voltage range in which the sodium current reactivates can yield late EADs.15 Mason et al16 elegantly showed that the less negative oscillations of the membrane potential could be suppressed with calcium channel blockers, whereas sodium channel blockers more easily suppress the more negative oscillations. Amiodarone, which has both calcium17 and sodium18 channel blocking properties, can consistently suppress both types of oscillations16; this property could well contribute to the relatively low incidence of EADs and TdP with amiodarone.19 Third, during the final part of repolarization, the sodium system recovers from inactivation, so that slowing this recovery will provide more time for incompletely recovered or slowed conduction. The latter is known to facilitate reentry arrhythmias.20 Finally, because not all cardiac APDs are identical, it is important that many potassium channels be open at the end of the action potential and early during diastole. This not only clamps the membrane potential closer to the potassium equilibrium potential but also reduces the tissue impedance, rendering activation due to current flow between cells at different potentials less likely.21 For these reasons, it is expected that prolongation of APD by triangulation will be more dangerous than prolongation of APD by extension of the plateau. Oscillations during the plateau are inherently less dangerous, because the system is refractory to conduction.

Most of the chemicals in the present series were selected for study on the basis of their potential to block the HERG channel. From the above, it is easy to conceive how blocking of the repolarizing currents during phase 3 would slow repolarization (triangulation), leading to stalling of repolarization and ultimately to EADs, TdP, conduction disturbances, reentry, and fibrillation. Some of the agents, however, were able to lengthen the APD without triangulation. Could interaction with the HERG channel possibly account for squared and stable prolongation of the APD? In theory, an agent could bind preferentially to the channel protein at positive potentials when the channel is in the inwardly rectifying configuration. Binding to the inwardly rectified channel could reduce its occasional openings during the plateau, thus maintaining a more positive plateau, until another current (eg, $I_{Ks}$) increases enough to initiate repolarization. As soon as inward rectification is removed at more negative potentials, the agent may dissociate from its receptor and thus allow for normal fast repolarization. Such state-dependent binding has been shown for many other channels.22–24 Of course, because many chemicals interacting with ion channels are quite promiscuous, ie, interact with many different ion channels, we cannot rule out the possibility that...
other channels could also be involved, but these considerations are beyond the scope of the present study.

The mechanism for development of instability must ultimately be electrophysiological in nature. Variability of APD has been ascribed to stochastic variations in the slowly inactivating sodium current, the delayed rectifier current, intracellular calcium transients, and reduced cellular coupling. In addition, reverse use-dependence would also be expected to be a potent contributor. Indeed, an ectopic without compensatory pause will elicit a short cycle length, which will in turn shorten the next APD. The shorter APD will be followed by a longer diastolic interval, eliciting a longer APD. An ectopic with compensatory pause will immediately be followed by a prolonged APD, then leading to the same oscillation. The steeper the restitution of APD versus diastolic interval, the more marked the oscillation is expected to become. Actually, it has previously been shown that when this slope exceeds unity, the system becomes predictably chaotic. In our subgroup analysis, reverse use-dependence, this could lead to complex instabilities. Ultimately, in places in which the currents exhibited widely varying APDs. Such potential differences result, simultaneously recorded action potentials frequently develop, it occurs in a spatially nonuniform fashion. As a reason, when an in vitro rabbit heart is used for safety assessment, instability of QT might provide a warning for impending proarrhythmia problems.

Clinical Implications
In the majority of cases, sudden cardiac death is the result of malignant ventricular tachyarrhythmias, including monomorphic VT, polymorphic VT, and ventricular fibrillation. The mechanisms underlying polymorphic VT most likely include abnormalities in ventricular repolarization, such as EADs and increased spatial dispersion of repolarization and functional reentry. Recent clinical studies showed a relation between increased beat-by-beat QT interval variability (instability) and increased risk for sudden cardiac death. The present study in perfused rabbit hearts shows a close relation between drug-induced instability of APD and proarrhythmia. In most cases, the APD variability preceded the proarrhythmia, which was most often polymorphic in nature. If patients at risk after a major cardiovascular event could be identified more effectively, the likelihood of sudden cardiac death probably could be reduced by proper initiation of therapy. The present animal study supports findings from earlier clinical studies indicating that instability of repolarization could serve as such an early indicator for increased risk of life-threatening arrhythmias.

Another implication of the present study is that increased temporal dispersion of repolarization may be an important factor for identifying patients at risk when therapy with repolarization-delaying agents (class III/class Ia) is initiated. This assumption is also supported by a clinical study with the Kl-blocking class III agent almokalant, in which it was demonstrated that an increased instability of QT characterized patients who subsequently developed TdP. If instability also commonly precedes proarrhythmia in patients who are sensitive to proarrhythmia induced by class III agents, then it might be possible to recognize some of the vulnerable patients by careful electrophysiological monitoring during their initial treatment. Similarly, if the electrophysiological substrate changes during therapy, instability of QT might provide a warning for impending proarrhythmia problems.

Most importantly, if the present results obtained in the isolated rabbit heart have an equivalent in patients, then QT prolongation is not a surrogate end point for sudden death. On the contrary, if the QT prolongation is well behaved (no instability, normal T wave, and no reverse use-dependence), then it is expected to be antiarrhythmic instead of proarrhythmic.

Shortcomings
An important observation is that when temporal instability develops, it occurs in a spatially nonuniform fashion. As a result, simultaneously recorded action potentials frequently exhibited widely varying APDs. Such potential differences between adjacent bundles would generate current flow. This current would in turn induce depolarization (where inward) and repolarization (where outward). Especially in the presence of reverse use-dependence, this could lead to complex instabilities. Ultimately, in places in which the currents became strong enough, they might induce depolarization-induced automaticity and contribute to the development of TdP. To fully answer these possibilities would require the use of many more recording sites.

Although 20 in vitro experiments could generally produce a ranking similar to that obtained by 149 in vivo experiments, the correlation was not perfect. Numerous reasons exist for these possible discrepancies. An important one is the fact that the proarrhythmia concentration-response curve is usually rather steep, so that small concentration differences between the 2 systems could lead to major differences. Rather than trying to provide a comprehensive list, one must assume that any system will always make occasional errors. For this reason, when an in vitro rabbit heart is used for safety analysis, it is mandatory that >1 experiment be done per chemical and that studies also be repeated in other species and with other tests. In addition, one should take into account that the rabbit heart appears to be very sensitive to class III-type problems. This may relate to the fact that the rabbit appears to have relatively little Kl to fall back on when Kl becomes blocked. Although great sensitivity can be a blessing, it could also erroneously lead to rejection of an excellent compound.

In hindsight, there are 3 improvements that could benefit studies like the present one. First, a systematic scan of various cycle lengths should be used. In the present study, we did this only in controls and in the highest drug concentration studied,
but this information was not automatically tabulated to allow cross-chemical comparison. We have seen occasions, however, when the cycle lengths of 300 and 1000 ms could be rigorously followed but intermediate cycle lengths exhibited EADs or even brief runs of TdP. Second, restitution curves might similarly be a good idea, because agents that render the slope of the restitution curve more positive over an extended diastolic interval are theoretically expected to promote chaos. Third, it might also be useful to measure the settling kinetics of a rhythm disturbance. Evaluation of the resulting instability might further improve the recognition power of agents that can destabilize the heart in a dangerous way.

Conclusions
APD prolongation is not necessarily associated with instability, triangulation, or proarrhythmia. Agents that lengthen the APD without inducing instability, triangulation, or reverse use-dependence are not proarrhythmic but rather antiarrhythmic in vitro. Furthermore, agents that induce less instability and triangulation in vitro are also less proarrhythmic in vivo (at least in the rabbit). Thus, instability, triangulation, and reverse use-dependence may be more important in predicting proarrhythmia than prolongation of QT. Whether this may also be extrapolated to humans will require additional studies.

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