Antiarrhythmic Effects of Potassium Channel Openers in Rhythm Abnormalities Related to Delayed Repolarization

Leif Carlsson, PhD; Christina Abrahamsson; Lissen Drews; and Göran Duker, PhD

Background. Earlier observations have indicated that repolarization-delaying agents may, under certain circumstances, have the propensity to induce polymorphous ventricular tachyarrhythmias (PVTs) (i.e., torsade de pointes). We have studied whether the potassium channel opener pinacidil and two of its pyridylecyanoguanidine analogues (P1075 and P1188) have any antiarrhythmic effects on clofilium-induced PVTs and triggered responses in rabbits in vivo and in vitro.

Methods and Results. Anesthetized rabbits were pretreated with propranolol (2 μmol/kg i.v.) and subsequently given a concomitant intravenous infusion of clofilium (63 nmol/kg/min for maximally 15 minutes) and the α₁-agonist methoxamine (70 nmol/kg/min). In vehicle-pretreated rabbits (n=19), clofilium invariably induced PVTs, which closely resembled torsade de pointes and were preceded by a marked prolongation of the QTU interval (27±2.4%, p<0.001). In a separate group of seven rabbits in which monophasic action potentials were recorded from the left ventricular endocardium, the tachyarrhythmia was preceded by deflections consistent with early afterdepolarizations (EADs) of the plateau repolarization phase of the monophasic action potentials. Intravenous administration of the pyridylecyanoguanidines in doses reducing mean arterial blood pressure by 25 or 50 mm Hg, respectively, was associated with a dose-dependent attenuation in the occurrence of clofilium-induced PVTs. In the pinacidil-pretreated rabbits (0.41 μmol/kg or 1.86 μmol/kg i.v.), the occurrence of PVTs was reduced from seven of seven rabbits to five of six and to three of seven rabbits (p=0.035 versus vehicle-pretreated controls), respectively. In rabbits pretreated with the low dose of P1075 (0.01 μmol/kg i.v.), PVT occurrence was reduced from six of six rabbits to two of six rabbits (p=0.030), whereas in six rabbits given the high dose of P1075 (0.13 μmol/kg), no PVTs appeared (p=0.001). When the sulfonylurea glibenclamide (10 μmol/kg i.v.) was administered to rabbits before P1075 (0.13 μmol/kg) was infused, clofilium induced PVTs in five of six rabbits (not significantly different from the incidence in the vehicle-pretreated rabbits). Pretreatment with P1188 (4.36 μmol/kg or 11.88 μmol/kg i.v.) caused a reduction in the occurrence of PVT from six of six rabbits to five of six and to none of six rabbits (p=0.001), respectively. In the six animals pretreated with the high dose of P1188 in which no clofilium-induced arrhythmias were elicited, glibenclamide (20 μmol/kg i.v.) was injected after the entire dose of clofilium had been administered. In these rabbits, premature ventricular systoles and PVTs appeared within a few minutes in five and four of the animals, respectively. In contrast to the pyridylecyanoguanidines, diltiazem pretreatment (0.9 μmol/kg i.v., decreasing arterial pressure by 50 mm Hg) did not attenuate PVT occurrence (five of six rabbits). Acute administration of P1075 (0.13 μmol/kg) during recurrent attacks of PVTs abruptly regularized the rhythm in 12 of 13 animals and diminished EADs observed in monophasic action potentials recorded from the left ventricular endocardium. In in vitro experiments, action potentials were simultaneously recorded from rabbit Purkinje fibers and ventricular muscle cells. Clofilium markedly prolonged action potential duration in Purkinje fibers but not in ventricular muscle cells, and eventually, bradycardia-dependent EADs and triggered activity were elicited. P1075 completely abolished EADs and triggered activity in all (six of six) experiments. Glibenclamide antagonized the suppressive effect of P1075; hence, EADs and triggered responses reappeared and resembled those present before P1075.

Conclusions. These results suggest that ATP-sensitive potassium channel activation can suppress rhythm abnormalities related to delayed repolarization and EADs and may provide a novel and useful intervention in the clinical occurrence of the acquired long QT syndrome. (Circulation 1992;85:1491-1500)

Key Words • repolarization-delaying agents • proarrhythmias • afterdepolarizations, early • torsade de pointes • pinacidil

Polymorphic ventricular tachyarrhythmia (PVT)—denoted torsade de pointes is occasionally observed in patients treated with repolarization-delaying agents and in patients with idiopathic long QT syndrome. A large body of circumstantial evidence suggests that triggered activity induced by bradycardia-dependent early afterdepolarizations (EADs), probably originating in the Purkinje fiber network, is a potential
mechanism underlying torsade de pointes.1,3,4 Among antiarrhythmic agents, quinidine is the most frequently cited culprit in torsade de pointes, although most class I A and III drugs have been reported to possess the propensity to induce the arrhythmia.1,2

In the clinical setting, “overdrive suppression” by means of rapid pacing or infusion of isoprenaline is the most effective therapeutic intervention in the case of torsade de pointes.1,2 Isoprenaline infusion, however, is contraindicated in patients with ischemic heart disease or hypertension and may be fatal in patients with ventricular tachyarrhythmias (VT) erroneously diagnosed as torsade de pointes. A new modality for pharmacological suppression of experimentally induced rhythm abnormalities related to prolonged repolarization was recently demonstrated in animal studies in which pinacidil and cromakalim, two potassium channel openers that increase potassium conductance, were found to effectively abolish EADs, triggered activity, and PVTs.5,7 Both pinacidil and cromakalim belong to a novel class of antihypertensive agents that are thought to act by causing hyperpolarization of smooth muscle cells, which in turn causes relaxation.8 However, there is evidence that, in addition to their effects on smooth muscle cells, potassium channel openers may alter potassium conductance in ventricular myocytes and thus shorten the action potential duration (APD) and reduce refactoriness.8–12 Evidence derived from patch-clamp electrophysiological studies and the ability of various antiadibetic sulfonylureas to block the effects of potassium channel openers suggest that the ATP-dependent potassium channel is the principal myocardial potassium channel affected.13

This study comprises a series of in vivo and in vitro experiments that were designed to examine the antiarrhythmic effects of pinacidil and two related pyridylcycanoquinuaines, P1075 and P1188, on clofibrate-induced PVTs, EADs, and triggered activity in rabbits. (A preliminary report of a part of this work has appeared in abstract form.14)

See p 1627

Methods

This study was approved by the local ethics committee on animal experiments in Gothenburg, Sweden.

In Vivo Experiments

Male New Zealand White rabbits (2.5–3.5 kg body wt) were used in this study. Anesthesia was initiated with methohexitol–sodium (Brietal 5 mg/kg) injected via a marginal ear vein and followed by infusion of α-chloralose (90 mg/kg, infusion volume 9 ml) administered for 20 minutes. No further anesthetic was needed. After tracheotomy, the animal was artificially ventilated with room air with a ventilator for small animals constructed in our laboratory. Blood gases and pH were kept within the physiological range by adjustment of tidal volume and respiratory frequency. Catheters were implanted into the right carotid artery and the jugular veins for recording of arterial blood pressure and infusion of drugs, respectively. Potassium levels in the arterial blood were estimated by means of a sodium–potassium analyzer (KNA1, Radiometer, Copenhagen, Denmark). Standard surface electrocardiograms (ECGs) (leads I–III, aVL, aVR, and aVF) were recorded on a Mingograph ink-jet recorder (Mingocard 7D, Siemens-Elema, Sweden), and arterial blood pressure and heart rate were recorded on a Grass polygraph (model 7D, Grass Instruments Co., Quincy, Mass.). In addition, the ECGs, blood pressure, and heart rate were recorded at predetermined intervals on a Compaq personal computer (Descpro 386s) at a sampling frequency of 500 Hz for sampling periods of 5 seconds. This personal computer was connected via an Ethernet local area network to a VAX-11/730 computer (Digital Equipment Corp., Maynard, Mass.). The final data processing was performed using the BIOLAB program, which is a program developed in our laboratory for acquisition and analysis of physiological signals measured in experimental animals.15

In some rabbits, a 4F bipolar contact electrode catheter (electrode distance, 3 mm) was advanced under fluoroscopic control through the left common carotid artery and positioned within the left ventricular cavity for recording of endocardial monophasic action potentials, which were recorded from a single endocardial site throughout the experimental period. Blood pressure was measured via a catheter inserted into the left femoral artery. Multiple ECG leads and endocardial monophasic action potentials were recorded on a Mingograph ink-jet recorder. The methods for analyzing the monophasic action potential have been described elsewhere.15,16

In a separate dose–range study, anesthetized rabbits received increasing intravenous bolus doses of pinacidil (from 0.1 to 5.0 μmol/kg, n=3), P1075 (from 0.001 to 1.0 μmol/kg, n=3), P1188 (from 0.1 to 10.0 μmol/kg, n=3), or diltiazem (from 0.01 to 5.0 μmol/kg, n=3) at 10-minute intervals, and blood pressure and heart rate were measured. From the dose–response curves obtained, doses of the various agents that produced a 25- or 50-mm Hg decrease in mean arterial blood pressure were defined by linear interpolation. To achieve the desired reductions in blood pressure of 25 or 50 mm Hg in the subsequent studies, pinacidil had to be injected at 0.41 or 1.86 μmol/kg, P1075 at 0.01 or 0.13 μmol/kg, P1188 at 4.36 or 11.88 μmol/kg, and diltiazem at 0.90 μmol/kg (reducing blood pressure by 50 mm Hg), respectively.

After a stabilization period, baseline recordings were obtained, and subsequently, propranolol (2 μmol/kg) was given intravenously to all rabbits. Propranolol was administered to avoid reflex tachycardia as a response to the reduction in blood pressure caused by the vasodilating agents. Previous studies in the anesthetized rabbit have shown that β-blockade per se does not influence the proarrhythmic response to clofibrate or the antiarrhythmic effect of pinacidil.4 Ten minutes later, a continuous infusion of the α1-agonist methoxamine (70 mmol/kg/min; infusion volume, 2 ml/kg/hr)5 was started and followed 5 minutes later by administration of pinacidil, P1075, P1188, or their vehicle. Five minutes later, clofibrate (63 mmol/kg/min; infusion volume, 0.1 ml/kg/min) was administered over a period of maximally 15 minutes (cumulative dose, 0.95 μmol/kg). After the start of the infusion of clofibrate, ECGs were continuously monitored on the ink-jet recorder and sampled on the computer once every minute, and the appearance of premature ventricular systoles and VTs was noted.
In a separate set of experiments, six rabbits were given glibenclamide (10 μmol/kg i.v. infused over 5 minutes) 10 minutes before the administration of the high dose of P1075 (i.e., 0.13 μmol/kg i.v.), which in turn was given 5 minutes before the start of the clofilium infusion as described above.

In six of the vehicle-pretreated rabbits, P1075 (0.13 μmol/kg) was injected and the infusion of clofilium was terminated (but not that of methoxamine) immediately after the appearance of the first episode of VT. Furthermore, in the seven vehicle-pretreated rabbits instrumented for recording of monophasic action potentials, an infusion of P1075 (1.3 μmol/kg/min) was started immediately after the first episode of VT.

Electrocardiographic Measurements

As soon as the infusion of clofilium had been initiated, surface ECGs were sampled for 5 seconds by the computer once every minute. All complexes during the 5-second sampling period were then analyzed, and the averaged ECG (lead I) was used for measurement of the QTU interval. Normally, a splitting of the T wave into two separate peaks (TU complex) could be seen in the ECG. The QTU interval was defined as the time between the first deviation from the isoelectric line during the PR interval until the peak of the TU wave.

In Vitro Experiments

In the in vitro study, male rabbits of the Russian strain (2.0–2.3 kg body wt) were used. The animal was anesthetized with pentobarbitone sodium (60 mg/kg i.v.), and the heart was excised and placed in a hyperkalemic and hypertonic solution of the following composition (in mM): NaCl 131, NaHCO3 18, CaCl2 2.5, MgCl2 0.5, NaH2PO4 0.9, KCl 27, glucose 55.

The right ventricular anterior papillary muscle with its free-running Purkinje fibers was excised and cut longitudinally. The preparation was mounted in a 2-ml organ bath (Steiert organ bath, Hugo Sachs Elektronik, March-Hugstetten, FRG) and superfused at a constant flow of 11 ml/min. After 1 hour, including the time for preparation, the solution was changed to a modified Tyrode's solution containing (in mM): NaCl 130, NaHCO3 18, CaCl2 1.8, MgSO4 0.5, NaH2PO4 1.8, KCl 4, glucose 5.5. Both solutions were continuously gassed with a mixture of 3% CO2/97% O2 to maintain a pH of 7.4, and the temperature was kept constant at 37°C.

After 30 minutes of superfusion with modified Tyrode's solution, stimulation of the preparation at 1 Hz (50% above threshold) was started (stimulator type 251/I, Hugo Sachs Elektronik). A stabilizing period of at least 2 hours was then allowed before the experimental protocol was initiated. A setup of two microelectrodes (filled with 3 M KCl; resistance, 2–4 MΩ) connected via Ag/AgCl junctions to high-impedance amplifiers (type 309, Hugo Sachs Elektronik) was used to make simultaneous recordings of transmembrane action potentials from both ventricular muscle cells and Purkinje fibers. The signals were recorded and analyzed in the way described for the in vivo experiments, with the exception of the sampling frequency, which was increased to 1,000 Hz. The amplified transmembrane potential signals were also recorded on a strip chart recorder (Graphitec WR 3600, Graphitec Corporation, Tokyo) at a paper speed of 100 mm/sec.

To mimic the conditions in the in vivo experiments in which clofilium was continuously infused into rabbits, an experimental approach was used in which the concentration of clofilium in the superfusate was continuously increased over time. After a period when control recordings were made, clofilium was continuously added to the reservoir containing Tyrode's solution flowing into the organ bath. With a certain volume in the reservoir and a known inflow of clofilium and rate of outflow of solution, the increase of the concentration of clofilium in the reservoir, and thereby in the organ bath, could be calculated and was set to 50 nmol/min. During the administration of clofilium, action potentials were recorded at various intervals until EADs appeared. When EADs arose, the addition of clofilium was stopped, and the clofilium concentration in the superfusate was subsequently maintained at that particular level. Immediately after the clofilium infusion was interrupted, superfusion with P1075, with the same rate of increase of concentration as for clofilium, was started. This superfusion was continued until the EADs disappeared and, as for clofilium, the concentration of P1075 in the reservoir was then maintained. Recordings were made as soon as the EADs had vanished. The superfusate now contained clofilium at a concentration that induced EADs in the absence of P1075 and P1075 at a concentration that suppressed the clofilium-induced EADs. Under these conditions, 10 μM glibenclamide was finally administered to the organ bath.

Statistical Analysis

Data are presented as mean±SEM and n indicates the number of observations. Analysis of variance (ANOVA) and Student's t test for unpaired or paired observations were applied when appropriate. The Fisher exact probability test was used to compare the frequency of VT induction. A probability value of less than 0.05 was considered statistically significant.

Drugs

Drugs used were clofilium tosylate (synthesized by Astra Hässle, Mölndal, Sweden), glibenclamide (Sigma, St. Louis, Mo.), methoxamine hydrochloride (Sigma), diltiazem hydrochloride (Sigma), pinacidil monohydrate (Løvens Kemiske Fabrik, Ballerup, Denmark), P1075 (Løvens), P1188 (Løvens), and propranolol hydrochloride (Sigma). All drugs were freshly prepared each day, and all doses in the text refer to bases of the compounds. Methoxamine, diltiazem, and propranolol were all dissolved in saline. Clofilium was dissolved in polyethylene glycol 300 and distilled water (13%/87%) and glibenclamide (50 mg) in 1 ml polyethylene glycol 400+1 ml ethanol+0.5 ml 1 M NaOH+2.7 ml distilled water or in dimethylsulfoxide (in vitro experiments). Stock solutions of the pyridylecyanoguanidine derivatives were dissolved in ethanol and distilled water (25%/75%), and appropriate dilutions were made with saline. Control experiments were performed with the solvent of the pyridylecyanoguanidines. This solvent did not have any significant influence on any of the hemodynamic or electrophysiological parameters measured.
Results

Characterization of Tachyarrhythmia In Vivo

In nineteen vehicle-pretreated rabbits, the concomitant infusion of clofilium and methoxamine was invariably (19 of 19) associated with the appearance of premature ventricular systoles, which, after a short lag phase in all animals, turned into PVT. The mean cumulative dose of clofilium administered at the time of the onset of the first premature ventricular systole was 0.4±0.05 μmol/kg, whereas the first PVT episode was observed after a mean cumulative dose of 0.6±0.05 μmol/kg. The first premature ventricular systole was preceded by a substantial lengthening of the QTU interval and RR interval (from 131±3.3 to 164±3.5 msec, p<0.001 and from 271±8.4 to 305±11.6 msec, p<0.001, respectively; n=19). In 17 of the 19 vehicle-treated rabbits, it was possible to determine the cycle length changes just before the onset of the first episode of the PVT. The tachyarrhythmia was initiated in a consistent mode with a premature ventricular beat followed by a postectopic pause and a subsequent supraventricular beat, which in turn was followed by a premature depolarization interrupting the T wave (Figure 1). The relations between the long (initiating) cycle length and the short cycle lengths are illustrated in Figure 2. The PVT, normally self-terminating, showed a typical “twisting morphology” with oscillating peaks of sequential QRS complexes (Figure 1).

In seven rabbits studied separately, clofilium induced a significant prolongation of the endocardial monophasic APD without affecting the rise time of the monophasic action potential. In Figure 3, the monophasic APD (at 90% repolarization) and the rise time of the monophasic action potential during the first 6 minutes of clofilium infusion (before premature ventricular sys-

toles appeared and became too frequent for reliable signal analysis) are illustrated. The first premature ventricular systole appearing after a mean cumulative dose of 0.6±0.1 μmol/kg was preceded by a lengthening of the monophasic APD from 126±3.7 to 188±7.8 msec. Furthermore, deflections of the late repolarization phase of the monophasic action potential both preceded (although not resulting in extrasystoles) and were prominent during the arrhythmia (Figure 4).

Effects of Pretreatment With Pyridylcyanoguanidines on Clofilium-Induced Polymorphous Ventricular Tachyarrhythmias

The effects of pretreatment with equihypotensive doses of pinacidil, P1188, and P1075 on clofilium-

![Figure 1](image1.png)

**Figure 1.** Representative electrocardiograms demonstrating initiation of torsade de pointes in an anesthetized rabbit given clofilium (63 nmol/kg/min i.v.). Tachyarrhythmia was induced after administration of a cumulative dose of 0.43 μmol/kg clofilium. Note characteristic pause-dependent mode of initiation (“short-long-short” sequence, S1 L S2) and undulating morphology of sequential QRS complexes.

![Figure 2](image2.png)

**Figure 2.** Graph shows relations between the short cycle (S1 and S2) lengths and the long cycle (L) length of clofilium-induced torsade de pointes whose full onset was recorded in 17 of the 19 vehicle-pretreated rabbits. Diagonal line represents the line of unity.

![Figure 3](image3.png)

**Figure 3.** Graph shows effects of clofilium (63 nmol/kg/min i.v.) on endocardial monophasic action potential (MAP) duration at 90% repolarization (left Y axis) and rise time of the upstroke of the monophasic action potential (right Y axis). Figure illustrates electrophysiological effects of clofilium measured until ventricular extrasystoles were too frequent and rendered reliable recordings impossible. Values are mean±SEM for seven rabbits. *p<0.05, **p<0.01, ***p<0.001 vs. predrug values.
induced PVTs were examined in 56 propranolol-pretreated rabbits. The doses of the various potassium channel openers, reducing mean arterial blood pressure by 25 or 50 mm Hg, were determined from separate dose–response experiments in anesthetized rabbits (see "Methods" for further details). The QTU interval recorded before infusion of clofilium was not significantly shorter in any of the groups of animals pretreated with potassium channel openers than in the corresponding interval observed in the vehicle-pretreated rabbits.

As described above, infusion of clofilium caused PVTs in all vehicle-pretreated rabbits, whereas pretreatment with the potassium channel openers dose-dependently attenuated the occurrence of PVTs. In the pinacidil-pretreated rabbits (0.41 μmol/kg or 1.86 μmol/kg i.v.), the occurrence of PVTs was reduced from seven of seven rabbits to five of six (p=0.46 versus vehicle-pretreated animals) and to three of seven rabbits (p=0.035), respectively (Figure 5A). In the rabbits pretreated with the lower doses of P1188 or P1075 (4.36 μmol/kg and 0.01 μmol/kg i.v., respectively), the PVT occurrence was reduced from six of six to five of six (p=0.50) and to two of six animals (p=0.030), respectively (Figures 5B and 5C, respectively). No premature ventricular systoles or PVTs were observed in rabbits pretreated with the high dose of P1188 (11.88 μmol/kg i.v., p=0.001 versus the PVT occurrence in the vehicle-pretreated animals). Likewise, a significant attenuation in the occurrence of PVTs was seen in rabbits pretreated with the high dose (0.13 μmol/kg i.v., p=0.001) of P1075 (premature ventricular systoles were observed in only one of six rabbits and PVTs were absent in all rabbits; Figure 5C). The lack of PVTs in these P1188- and P1075-pretreated rabbits was observed despite a significant prolongation of the QTU interval by clofilium. After administration of the entire dose of clofilium (0.95 μmol/kg i.v.), the QTU intervals were increased by 33±6.6% (P1188) and 34±9.0% (P1075), respectively. A QTU prolongation that did not differ significantly from the one (27±2.4%) observed immediately before the first premature ventricular systole in the vehicle-pretreated animals.

Effects of Acute Intervention With Pyridylcyanoguanidines on Clofilium-Induced Polymorphous Ventricular Tachyarrhythmias

In six vehicle-pretreated rabbits, the infusion of clofilium was terminated and P1075 (0.13 μmol/kg) was injected immediately after the appearance of the first run of VT. Within 15 seconds, a prompt suppression of
the arrhythmia was observed in five animals. In the nonresponding rabbit, P1075 was injected after the abrupt initiation of a sustained VT. In the seven rabbits instrumented for recording of endocardial monophasic action potentials, P1075 was slowly infused into animals with VT until the rhythm was normalized. Successful regularization of rhythm was observed in all animals after a mean cumulative dose of 97±13.7 nmol/kg P1075. Suppression was associated with a nonsignificant alteration in the monophasic APD and RR interval (from 188±7.8 msec and 317±18.7 msec immediately before the appearance of the first premature ventricular systole to 175±19.0 msec and 322±24.6 msec 1 minute after infusion of P1075, respectively). Five minutes after the infusion of P1075 (all rabbits still in sinus rhythm), the monophasic APD was 174±9.0 msec.

**Effects of Glibenclamide and P1075 on Clofilium-Induced Polymorphous Ventricular Tachyarrhythmias**

Sulfonylurea glibenclamide (10 μmol/kg i.v.) was administered to six rabbits before the high dose of P1075 (0.13 μmol/kg i.v.) was infused. In this group of rabbits, clofilium lengthened the QTU interval from 155±4.2 msec to 208±18.3 msec (recorded immediately before the first premature ventricular systole appeared). PVTs were induced in five of the six animals, a PVT occurrence not significantly different from the one observed in the vehicle-pre-treated animals (Figure 5C). In the group of six rabbits pretreated with the high dose of P1188 and in which no clofilium-induced dysrhythmias appeared (Figure 5B), glibenclamide (20 μmol/kg i.v.) was given as a bolus injection after the entire dose of clofilium had been administered. In these six rabbits, premature ectopic beats appeared within a few minutes in five animals and PVTs in four.

**In Vitro Results**

The values of the baseline APD (at 75% repolarization) in ventricular muscle cells and in Purkinje fibers were 119±4.2 msec and 284±5.2 msec, respectively. The effects of an increasing concentration of clofilium on the APD in ventricular muscle cells and in Purkinje fibers are shown in Figure 6. During superfusion with clofilium, the APD in Purkinje fibers showed a gradual prolongation until EADs eventually developed (Figure 7, panels A–C). In four of six preparations, EADs propagated and initiated triggered responses in ventricular muscle cells (Figure 7C). Clofilium concentration at the time when EADs first appeared in Purkinje fibers was 1.1±0.17 μM (n=6), and maximum APD in Purkinje fibers preceding EADs was 603±35.5 msec (p<0.001 versus pre–drug duration). The APD in the ventricular muscle cells was not significantly affected by clofilium. Hence, the action potentials recorded simultaneously with the longest action potentials in Purkinje fibers had a duration of 124±5.2 msec.

Administration of P1075 was associated with a shortening of the clofilium-induced APD prolongation and a gradual disappearance of clofilium-induced EADs. The APD in Purkinje fibers was, however, still markedly longer than it was before the administration of clofilium (Figure 7D). The final concentration of P1075 at which EADs disappeared was 1.7±0.18 μM (n=6), and the APDs in ventricular muscle cells and Purkinje fibers recorded immediately afterward were 94±12.1 msec and 434±57.1 msec, respectively.

Addition of glibenclamide (in the presence of clofilium and P1075) resulted in a rapid prolongation of the APD in Purkinje fibers in four of the six preparations (to 615±67.3 msec), and within 2.5–5.5 minutes, the duration was prolonged markedly and EADs eventually reappeared (Figure 8).

To further confirm the characteristic features of clofilium-induced EADs, the influence of the stimulation frequency was examined in three of the six experiments. An increase in the stimulation frequency was associated with a gradual suppression and finally a disappearance of the clofilium-induced EADs. Furthermore, when the stimulation was interrupted for various time periods, the number of EADs after the first post–pause-stimulated action potential increased with the length of the pause, i.e., the APD was further increased by the reduction in frequency and more EADs developed during the plateau phase (Figure 9).

**Discussion**

Observations from this study have demonstrated that ventricular rhythm abnormalities related to delayed repolarization can effectively be prevented or suppressed by the potassium channel opener pinacidil and its related pyridylcyanoguanidine derivatives P1075 and P1188. The main part of the study was performed in an animal model of the acquired long QT syndrome in which action potential–prolonging agents induce a form of PVT closely resembling clinical torsade de pointes. Hence, the tachyarrhythmia was preceded by a marked prolongation of the QTU interval as well as lengthening of the endocardial monophasic APD, a distinct prominent U wave, deflections in the late repolarization phase.
of the monophasic action potential, a consistent mode of initiation ("short-long-short sequence"), and the typical undulating morphology of sequential QRS complexes. Although the antiarrhythmic effect of the potassium channel openers may seem predictable in view of their properties of increasing membrane potassium conductance (thereby shortening the APD), it was of interest to note that pretreatment with P1075 or P1188 prevented PVTs despite a clofilium-induced QTU prolongation that did not differ significantly from the lengthening observed in the vehicle-pretreated rabbits. Similarly, acute intervention with P1075 caused a prompt regularization of rhythm with only a modest reduction in the clofilium-induced monophasic action potential lengthening. Moreover, in isolated Purkinje fibers, P1075 caused a suppression of EADs and triggered activity despite a substantial clofilium-induced prolongation of the APD. Concordant results were recently presented by Fish and coworkers\(^5\) in a study in which EADs and triggered activity were elicited in canine Purkinje fibers during superfusion with quinid-ine, cesium, or mexitilide. In that study, two structurally unrelated potassium channel openers, pinacidil and cromakalim, were demonstrated to abolish rhythm abnormalities related to delayed repolarization at concentrations that did not reverse the primary drug-induced action potential prolongation.

A comparison of the cardiac and vascular effects of various putative potassium channel openers was made by Steinberg and colleagues.\(^9\) Their study indicated that among the different pyridyldianoguanidines examined, pinacidil had the most pronounced vascular selectivity, whereas P1188 showed the opposite, i.e., cardiac exceeded vascular selectivity. These results formed the
basis for our selection of pinacidil, P1075, and P1188 (with a wide range of vascular to cardiac selectivity ratios) for in vivo studies on suppression of tachyarrhythmias related to abnormal repolarization. The results obtained are in agreement with the findings of Steinberg and coworkers inasmuch as P1188 and P1075, being more cardioselective than pinacidil, were somewhat more effective at a given degree of hypotension than pinacidil in preventing clofilium-induced PVTs.

The mechanisms underlying torsade de pointes in patients with the acquired (pause-dependent) long QT syndrome are still poorly understood, although it is well recognized that torsade de pointes may occur in the setting of delayed repolarization.1-4 The existing clinical and experimental evidence suggests that dispersion of repolarization and/or EADs may constitute the electrophysiological substrate(s) of torsade de pointes.1 In the present study, both these phenomena were observed. Hence, deflections in the repolarization phase consistent with EADs were observed both in monophasic action potentials recorded from the left ventricular endocardium of the anesthetized rabbit and in transmembrane action potentials recorded from the isolated rabbit Purkinje fiber. Such deflections were never observed in action potentials recorded from isolated ventricular muscle cells, supporting a site of origin of EADs within the Purkinje fiber network. On the other hand, EADs appearing in Purkinje fibers occasionally propagated to the ventricular cell, in which a triggered response was initiated. This finding contrasts the lack of extrasystoles as a result of the deflections in the repolarization phase of the monophasic action potential recorded in vivo. At present, we do not have a clear explanation for this discrepancy between the in vivo and the in vitro findings. An indirect evidence of a linkage between the EADs and torsade de pointes in the concomitant disappearance of the EADs and the suppression of the clofilium-induced torsade de pointes (or the triggered activity in vitro) after acute intervention with P1075. Sasyuk and colleagues17 recently pointed out the fact that EADs and triggered activity may occur at multiple sites within the Purkinje system, which may explain the polymorphic nature of torsade de pointes. Furthermore, it was recently demonstrated that injection of anthopleurin-A into anesthetized dogs gave rise to EADs and triggered activity. EADs were more frequent and of higher amplitude in endocardial than in epicardial monophasic action potentials, a finding that may reflect a mixed electrical activity of Purkinje fibers and subendocardial muscle fibers.3,18 From our in vitro studies, it was obvious that clofilium caused a substantial prolongation of the APD in the Purkinje fiber, whereas the duration in the ventricular muscle cell was almost unaffected, thus causing an increased dispersion of repolarization. Under conditions of increased dispersion of repolarization, which may be a consequence of treatment with repolarization-delaying agents, areas with different APDs occur, and electrical gradients are thus generated. In such a situation, current can flow from structures with lower membrane voltages to structures with higher membrane voltages and thus electrotonically depolarize the latter structure, a phenomenon denoted “prolonged repolarization-dependent reexcitation” by Brugada and Wellens.19 Data were recently presented in support of the hypothesis that QT interval dispersion reflects differences in myocardial repolarization times and that such a QT dispersion may distinguish between patients susceptible to ventricular arrhythmias and patients without arrhythmias.20

An indication of an increased dispersion of repolarization was also found in the present study, in which clofilium caused a marked increase in the amplitude of the U wave. The origin of the U wave has been a subject of discussion and dispute for a long time. One hypothesis relates the U wave of the surface ECG to EADs,5,21 whereas Hoffman and Cranefield22 suggested that the U wave is a manifestation of the repolarization of Purkinje fibers. This latter hypothesis has been questioned because the small mass of Purkinje fiber network should not be able to generate enough current during the repolarization process to be detected on the standard ECG. Recently, however, evidence was presented in support of the existence of a unique population of cells (termed M cells) located deep in the subepicardial layer of the canine ventricle.23,24 In terms of action potential characteristics, these cells closely resembled Purkinje fibers, with the exception that phase 4 depolarization was never observed, and they did respond to repolarization-delaying agents with a substantial prolongation of the APD and the eventual appearance of EADs. Hence, it is possible that the mass of the particular subepicardial cells can add to the mass of Purkinje fibers and contribute to the genesis of the U wave.

Antiarrhythmic activity as well as proarrhythmic or profibrillatory effects have been reported for structurally unrelated potassium channel openers such as nicorandil, pinacidil, and cromakalim in various experimental models. In canine or sheep Purkinje fiber, cromakalim shortened the APD and suppressed automatic discharge induced by norepinephrine, barium, or strophanthidin. Furthermore, cromakalim was found to reduce or abolish oscillatory potentials induced by high [Ca2+]0.25 Likewise, in canine Purkinje fibers, nicorandil antagonized abnormal pacemaker activity induced by barium or low [K+].26 In a dog model of subacute myocardial infarction, pinacidil exhibited antiarrhythmic effects when examined 22-24 hours after a two-stage coronary artery ligation, an action that could not be explained by overdrive suppression of ventricular rhythm because β-blockade did not modify the response.27 However, in a model of ouabain-induced arrhythmias, no antiarrhythmic effects of pinacidil could be documented. In contrast to the studies demonstrating suppressive effects of potassium channel openers on arrhythmias of various origins, other studies have pointed out the fact that such agents can actually induce or contribute to the genesis of tachyarrhythmias. In isolated canine Purkinje fibers, high concentrations of pinacidil exacerbated barium-induced automaticity, and in some preparations, episodes of spontaneous tachycardia developed.28 Furthermore, pinacidil has also been demonstrated to produce a marked dispersion of repolarization within epicardial tissue and also between epicardial and endocardial canine tissue, which in turn augmented reentrant arrhythmias.29 Chi and coworkers10 recently demonstrated that, in the conscious dog, pinacidil may exert a profibrillatory effect in the postinfarcted heart during superimposition of an acute ischemic event. It was hypothesized that the increased tendency to develop ventricular fibrillation was a con-
sequence of a pinacidil-induced attenuation of ventricular refractoriness.

Despite the dissimilarity of their chemical structures, most potassium channel openers appear to mediate their myocardial effects by opening plasmalemma ATP-regulated potassium channels. Mammalian heart cells from various species have high-affinity receptors for antidiabetic sulfonylureas such as glibenclamide, an established blocker of the ATP-regulated potassium channel. Our data indicate that the antiarrhythmic effects of pinacidil and its derivatives are mediated via activation of the ATP-dependent potassium channel. In the anesthetized rabbit, pretreatment with glibenclamide antagonized the antiarrhythmic effect of P1075 and, furthermore, in rabbits given P1188 and in which cloflium did not initiate tachyarrhythmias, infusion of glibenclamide led to recurrent episodes of tachyarrhythmias. These observations were confirmed in the in vitro recordings in which glibenclamide reversed the suppressive effects of P1075 on cloflium-induced EADs and triggered activity. Fish and colleagues suggested that the major antiarrhythmic principle of potassium channel openers in arrhythmias related to delayed repolarization is an acceleration of repolarization, which may inhibit the depolarizing current responsible for the triggered response or increase the current needed to elicit the afterdepolarization and the triggered response. Evidence in support of this hypothesis was recently put forward by Spinelli and others, who demonstrated that the depolarizing current needed to elicit an EAD was markedly increased by pinacidil; furthermore, in single canine myocytes, pinacidil was found to abolish EADs caused by BAY K 8644 or ketanserin. Vasodilation and/or altered loading conditions may be referred to as an alternative explanation, because administration of the potassium channel openers in the in vivo experiments was associated with a substantial fall in blood pressure. Hansen and others recently stressed the importance of mechano-electrical feedback for induction of arrhythmias in globally dilated or dyskinetic ventricles. However, the lack of effect of diltiazem in the present study suggests that the antiarrhythmic effects of potassium channel openers are independent of changes in load or dilatation.

Conclusions

Our study indicates that EADs, triggered activity, and increased dispersion of repolarization may be important factors contributing to torsade de pointes associated with acquired long QT syndrome. The reliability of the rabbit treated with class III antiarrhythmic agents as a useful experimental model of acquired long QT syndrome has been additionally confirmed. Furthermore, the results demonstrate that activation of ATP-dependent (glibenclamide-sensitive) potassium channels can suppress rhythm abnormalities related to delayed repolarization and may be a novel therapeutic approach in the treatment of torsade de pointes.

Acknowledgment

We wish to acknowledge Dr. Ian Ahnfelt-Ronne, Lovens Kemiske Fabrik, Ballerup, Denmark, for the generous gift of the potassium channel openers.

References


Antiarrhythmic effects of potassium channel openers in rhythm abnormalities related to delayed repolarization.
L Carlsson, C Abrahamsson, L Drews and G Duker

_Circulation_. 1992;85:1491-1500
doi: 10.1161/01.CIR.85.4.1491

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/85/4/1491

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/