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Voltage- and Time-Dependent Block of the Delayed K^+ Current in Cardiac Myocytes by Dofetilide

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ABSTRACT

The delayed K^+ current (i_K) and its change by dofetilide was studied in single myocytes from the guinea pig and rabbit heart using the two-electrode voltage clamp technique. In rabbit myocytes, i_K consisted of only one component (K_r), which developed for moderate depolarizations and with a fast time course. In guinea pig myocytes, activation consisted of a rapid and a slow component, and the latter (K_s) only became manifest for depolarizations positive to 0 mV. K_s was resistant to block by dofetilide. K_r, however, was very sensitive: K_d 3.9×10^{-9} M, Hill coefficient 2.0 ($n = 5$). The effect was voltage-dependent block increasing at depolarized levels. Block development was time dependent and occurred in two phases: a first fast and voltage-dependent phase was followed by a second much slower phase (time constant of 4.4 ± 0.48 sec ($n = 11$). Recovery from block was slower as the membrane potential became more negative. This resulted in the absence of a steady-state frequency-dependent effect at negative membrane potentials. It is concluded that dofetilide is an efficient blocker of the fast component of i_K. The block, as well as recovery, are voltage and time dependent. Block is greater at more depolarized levels, recovery is slower at more hyperpolarized levels.

Reentry, which is one of the main mechanisms in the genesis of cardiac arrhythmias, is based on the existence of slow conduction in combination with a short refractory period. Blocking conduction in the abnormal reentry pathway or prolonging the refractory period will be antiarrhythmic. Reducing conduction velocity in normal tissue, however, may become arrhythmogenic, and the inherent arrhythmogenic properties of agents that block the Na^+ current have been emphasized by the CAST study (1989). The question, thus, whether to reduce conduction or to prolong refractoriness, is important, and compounds that prolong the action potential duration by inhibition of the i_K currently receive renewed attention (Colatsky *et al.*, 1990; Hondeghem and Snyders, 1990). In contrast to substances blocking the Na^+ current known for their negative inotropic effects, the compounds which block K^+ current may have an advantageous effect on contractility.

The present article presents a voltage clamp analysis of changes in ionic currents caused by dofetilide (UK 68798). The chemical structure has been published by Gwilt *et al.* (1991). The molecule can be considered a sotalol derivative with two methane sulphonamide groups at either end of the molecule. The compound has been shown to increase the action potential duration and the effective refractory period in canine ventricular muscle and Purkinje fibers without affecting resting potential, action potential amplitude or upstroke velocity. In guinea pig ventricular myocytes, the i_K was inhibited in a concentration-dependent way (Gwilt *et al.*, 1991). In the present article, more detailed information is provided about changes in ionic currents, with special emphasis on the voltage- and use-dependent effects, on the development of block during the depolarization and on the recovery phenomena at rest. Information on the kinetics of block and unblock of compounds active on i_K is nonexistent except for quinidine (Roden *et al.*, 1988; Furukawa *et al.*, 1989; Balser *et al.*, 1991). The present study also includes an analysis of dofetilide on other currents and a comparison of the effects on i_K in rabbit and guinea pig myocytes. The reason for such a comparison is the complex nature of the i_K current in the guinea pig. Sanguinetti and Jurkiewicz (1990) have shown that the delayed K^+ current in this preparation is composed of a rapidly activating component, i_{Kr}, and a slowly activating component, i_{Ks}; the latter component is less sensitive to blockade by E 4031.

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Materials and Methods

Cell preparation. Single ventricular myocytes of the guinea pig were dissociated by enzymatic dispersion, following a procedure described in detail previously (Tytgat *et al.*, 1990). In brief, the heart was quickly removed after decerebration of the animal and was mounted

ABBREVIATIONS: i_K, delayed K^+ current; i_{Kr}, rapidly activating K^+ current; i_{Ks}, slowly activating K^+ current; i_{Ki}, inward rectifier K^+ current; i_{to}, transient outward current; i_{Cl}, chloride current; HEPES, N-hydroxyethyl-piperazine-N′-2-ethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)N,N′-tetraacetic acid.

on a Langendorff perfusion system. The aorta was cannulated and the heart was perfused at 37°C with: 1) Ca^{++}-free standard solution (see solutions) for 5 min; 2) Ca^{++}-free standard solution containing 35 mg/50 ml collagenase A (Boehringer Mannheim, Germany) and 6.5 mg/50 ml protease XIV (Sigma Chemical Co., St. Louis, MO) for 5 min; and 3) 0.2 mM Ca^{++}-containing solution for an extra 10 min. After the isolation of cells, they were stored at room temperature in HEPES-Tyrode until used. For isolation of rat myocytes, phase 2 was prolonged to 35 min. For isolation of rabbit myocytes, phase 1 (10 min) was followed by perfusion for 15 min with a solution containing collagenase, and subsequently by 35 min with the solution containing collagenase and protease. In order to prepare single Purkinje cells, phase 1 was interrupted after 3 min, the rabbit ventricle was opened and Purkinje fibers were removed. Digestion of the tissue was then continued in the same solution until enough cells were obtained (Scamps and Carmeliet, 1989). A limited number of experiments were performed on multicellular Purkinje fibers; they were dissected from the right and left ventricles of rabbit heart.

Measurements. Aliquots of cells were allowed to settle on the glass bottom of a tissue bath (volume < 0.5 ml) and then superfused (3 ml/min, 37°C) with buffer solution. The two-electrode voltage clamp (Axoclamp 2-A amplifier, Axon Instruments, Foster City, CA) was applied using suction pipettes with resistance ranging from 2 to 5 megohms. P-clamp software (Axon Instruments) was used to generate voltage-pulse protocols and to acquire and analyze data. In some experiments, currents were directly recorded on a Gould Brush recorder and analyzed manually. In Purkinje fibers, the two-electrode voltage clamp (3 M KCl microelectrodes) was used on short segments obtained by crushing the fibers with a wire grid (Mubagwa and Carmeliet, 1983).

Solutions. The standard external solution contained, in mM: NaCl 137.6, KCl 5.4, $MgCl_2$ 0.5, $CaCl_2$ 1.8, HEPES 11.6 and glucose 5, and NaOH was added to pH 7.4. For cell isolation, $CaCl_2$ was omitted. When Cl^- current was measured, KCl was replaced by CsCl. In the experiments on Purkinje fibers, the bathing solution contained $NaHCO_3$ (24 mM) and was gassed with 95% O_2/5% CO_2.

Intracellular solution contained, in mM: KCl 120, $MgCl_2$ 6, $CaCl_2$ 0.154, Na_2ATP 5, EGTA 5 and HEPES 10, with KOH added until pH 7.2. In the Cl^- current experiments, the pipette solution contained: Cs aspartate 110, tetraethylammonium-Cl 20, CsCl 10, NaCl 10, $MgCl_2$ 1, K_2ATP 1, EGTA 5 and HEPES 5, and CsOH was added until pH 7.2.

Drugs. Dofetilide (Pfizer, Sandwich, GB) was prepared as a stock solution (10^{-3} M) by dissolving the drug in distilled water acidified by HCl to pH 3. Nisoldipine (Bayer, Leverkusen, Germany) was used in a final concentration of 0.2×10^{-6} M prepared from a stock solution of 10^{-2} M in dimethyl sulfoxide. Isoproterenol (Winthrop, New York, NY; stock solution 10^{-3} M) was added in a concentration of 10^{-6} M.

Statistics. Results are expressed as mean values ± S.E.

Results

i_K in Rabbit Ventricular Myocytes

Activation: Voltage dependency and concentration effects. Activation of i_K was studied by applying voltage clamp steps from a holding potential of −50 mV to different depolarizing levels. Tail currents were measured on return to the holding potential. Examples of currents during the depolarizing step and of the tails on return to the holding potential are given in figure 1 for control conditions and in the presence of 5×10^{-9} and 10^{-6} M dofetilide.

During the depolarizing clamp, outward current increased with time, and the change was greater and faster with a larger depolarization. The outward tail current also increased with depolarization. In the presence of the drug, time-dependent currents and tail currents decreased or were completely absent at the high concentration of 10^{-6} M. In this particular experiment, under the conditions used (*i.e.*, nisoldipine present to block i_{Ca}), all time-dependent current during the depolarizing clamp is blocked. It must be added, however, that this was only seen in 35% of the cells used (n = 33); in 39 and 26%, respectively, of the cells, a moderate to pronounced i_{to} was present and not blocked by the drug.

Quantitative data for tail currents obtained in six rabbit ventricular cells are summarized in figure 2. Under control conditions, the outward tail current increased with depolarization and saturated for depolarizations to +10 mV. Half maximum was obtained at −13.7 ± 1.4 mV (n = 6), and the slope was 4.9 ± 0.5 mV. Addition of 4.5×10^{-9} M of the drug resulted in a decrease of the tail current amplitude that was clearly voltage dependent, as the suppression became more pronounced with greater depolarization. No block was observed at −20 mV; inhibition was 30% at −10 mV and became 50% at 0 mV and more depolarized levels. This means that the normalized activation curve was shifted in the hyperpolarized direction. The shift amounted to −5.6 ± 0.6 mV. Similar results were obtained in four rabbit Purkinje fibers. Compared to single ventricular cells, the activation curve under control conditions was more to the left: half maximum was seen at −29 mV and the slope was 7.7 mV. Block by dofetilide (10^{-8} M) was also voltage dependent: 45% at −40 mV and 63% at 0 mV.

The blocking effect was dependent on concentration. Increasing drug concentrations were applied in a cumulative way, with each concentration applied for 10 min. The decrease in tail current as a function of drug concentration is shown in figure 3. The theoretical curve was drawn on the basis of the equation $[1 + ([D]/K_m)^n]^{-1}$ with a Hill coefficient n of 2,0 and a K_m of 3.9×10^{-9} M.

Use dependency. The notion of use-dependent block has been proposed to explain the increase of block of Na^+ or Ca^{++} currents upon repetitive stimulation. Information on use dependence for drugs acting on i_K is scarce and even controversial (Roden *et al.*, 1988; Furukawa *et al.*, 1989; Balser *et al.*, 1991). Figure 4 illustrates the results of an experiment in which a series of short (200 msec) depolarizing clamps up to −10 or +10 mV from a holding potential of −50 mV was applied at a repetition frequency of 1 Hz. Under control conditions (A and B), the current during the depolarizing pulse as well as the outward tails rapidly increased with successive depolarizations to a saturating value. In steady state, the amount of activation during the pulse equals the amount of deactivation during the time at the holding potential. In the presence of the drug, 3×10^{-9} M (C and D) summation of tails was small or absent, and was followed by a slow decrease. Note also that the first tail in the presence of the drug was not changed for pulses up to −10 mV (C), but was already markedly reduced for pulses up to +10 mV (D), in accord with a voltage-dependent fast block.

In eight preparations, use-dependent block was induced by clamp pulses of 300 msec to +10 mV followed by 200 msec at −50 mV (to measure the tail) and 500 msec at −75 mV repeated at a frequency of 1 Hz. After 1 min the tail decreased to 0.49 ± 0.05 and in steady state (2–3 min) to 0.36 ± 0.04 (see fig. 7).

Development of block. *Is the drug blocking channels in the rested state?* The observation that the drug did not reduce tail currents for moderate depolarizations (fig. 2 and fig. 4) already strongly suggests that it does not bind to channels in the rested state. In order to further test this hypothesis, the membrane was held at −75 mV while the drug was added in a concentration of 3×10^{-9} M. After 5 min of incubation, which is a sufficient time to exchange completely the extracellular space, block was

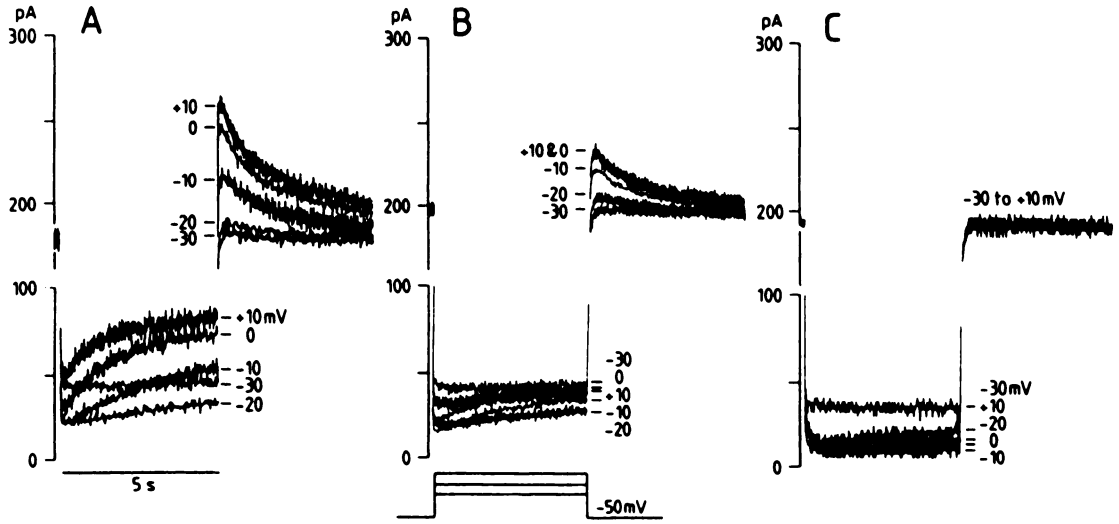

Fig. 1. Examples of current records during and after voltage steps to different depolarized levels indicated in the figure from a holding potential of −50 mV. The duration of the depolarizing step was 5 sec. Records are shown for control conditions (A) and in the presence of two concentrations of dofetilide (5×10^{-9} and 10^{-6} M, B, C). Rabbit ventricular myocyte. Two suction pipette voltage clamp. Temperature: 37°C.

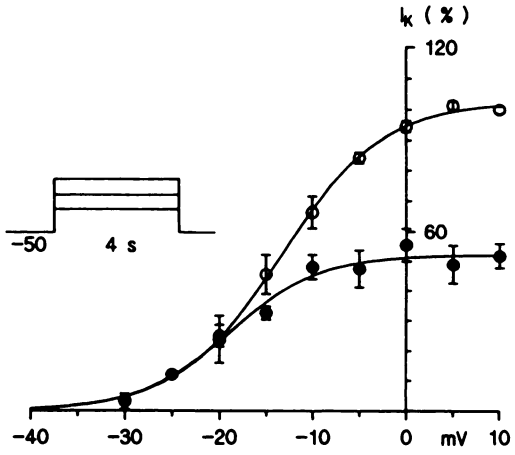

Fig. 2. Activation curve for i_K in six rabbit ventricular myocytes and its change by dofetilide (4.5×10^{-9} M). Relative amplitude of tail currents obtained for clamps of 4 sec duration from a holding potential of −50 mV as a function of test potential. Amplitude of tail current at +10 mV was taken as 100%. Mean values ± S.E. The block by the drug is voltage dependent, increasing with depolarization.

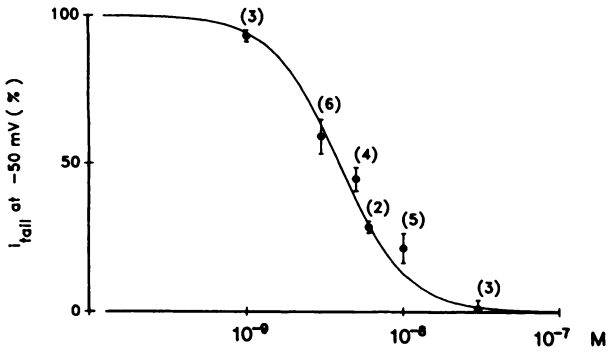

Fig. 3. Concentration-response curve. Relative block was estimated by measuring the decrease in tail current amplitude for clamp depolarizations of 4 sec duration to 0 mV. The theoretical graph is based on the equation $[1 + ([D]/K_m)^n]^{-1}$, with $n = 2$ and $K_m = 3.9 \times 10^{-9}$ M; rabbit ventricular myocytes.

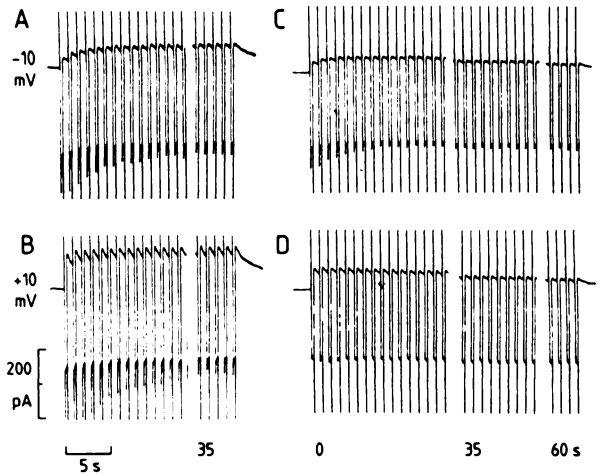

Fig. 4. Illustration of use-dependent block induced by 3×10^{-9} M dofetilide. Currents obtained for 0.2-sec depolarizations to −10 mV and +10 mV, applied at a frequency of 1 Hz, are shown. Holding potential: −50 mV. Panels on the left (A and B) in control condition, on the right (C and D) in presence of the drug. Under control conditions, tail currents show summation with repetition of the clamp depolarization. In the presence of the drug, this summation is less marked and is even reversed, showing a decline in tail with repetition; rabbit ventricular myocyte.

measured by applying a test depolarization. This test consisted of a 300-msec depolarization to +10 mV, followed by 200 msec at −50 mV to estimate the tail current amplitude and a return to −75 mV. In comparison with the tail under control conditions in the absence of the drug, tail amplitude was reduced to 0.84 ± 0.03 ($n = 8$) (see fig. 7).

Because the concentration used was sufficient to cause about 50% block at this test potential, the result suggests relatively little affinity of the closed channel at −75 mV for the drug. The observed decrease of 16% can indeed be explained by fast binding of the drug to the open channel occurring during the test depolarization of 300 msec (see next section and fig. 5).

Block development during depolarization. From a theoretical point of view, use dependence can be explained as being caused by an increase in affinity of the channel for the drug when the

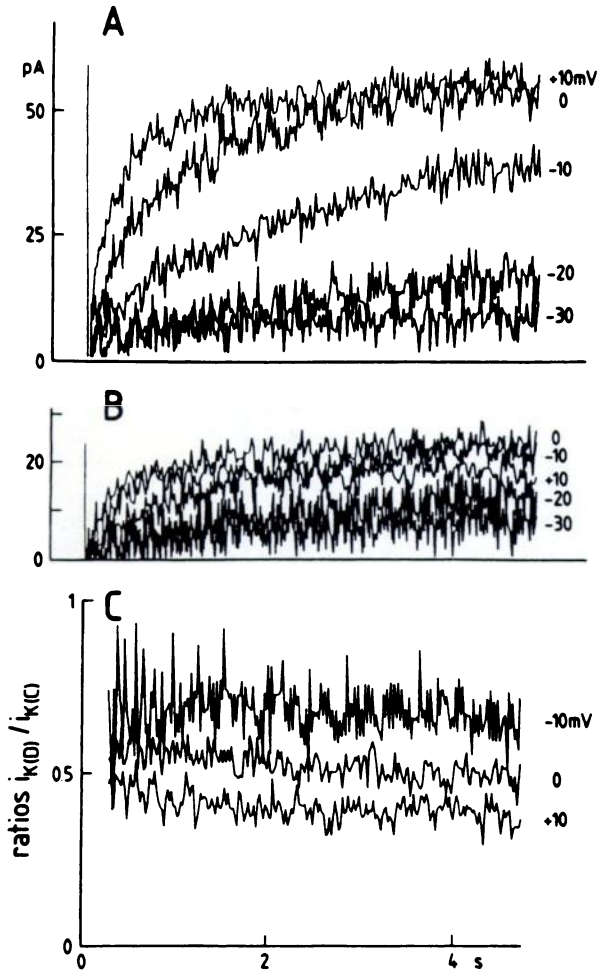

Fig. 5. Open-state block development estimated by current change during the depolarizing clamp (same experiment as in fig. 1). In A, difference between absence and presence of 10^{-6} M; in B, difference between 5×10^{-9} and 10^{-6} M. A represents the total drug-sensitive current, B the I_K current remaining in 5×10^{-9} M. Examples are shown for different levels of depolarizations indicated in the figure. C, Ratios of the current in the presence of 5×10^{-9} M, $i_{K(D)}$ and the total drug-sensitive current $i_{K(C)}$. A value of 1.0 means absence of block. Block is composed of an early, quasi-instantaneous block, which was voltage dependent, followed by a second slow phase with a time constant in the order of seconds; rabbit ventricular myocyte.

channel is activated. The time course of block development was studied either by analyzing the change in current during depolarizing steps or by measuring the change in tail current after depolarizing steps of different duration. For this type of experiment, cells with no or moderate i_{to} were selected.

Current during depolarizing pulses. Experiments in which the delayed K current was measured in the control condition and in the presence of an intermediate and a high concentration of the drug (see fig. 1) can be used to isolate the delayed K current from the total current and to measure its change by the drug. By subtracting the current remaining in the presence of a high concentration (10^{-6} M) from the current in the absence of the drug, a "pure" i_K current (reference current) without contamination by other currents can be obtained (Sanguinetti and Jurkiewicz, 1990). This reasoning is correct insofar as dofetilide does not affect other currents. An effect on the L-type Ca^{++} current was excluded by adding 10^{-6} M nisoldipine to the experimental solutions. In a latter section, it will be shown further that dofetilide had no effect on i_{to}, i_{K1} or i_{Cl}. Examples of i_K currents obtained by this procedure are given in figure 5A. As can be seen from this figure activation of the difference current is faster and greater when the depolarizing step is greater. Time constants for activation in this example were 150 msec at +10 mV, 374 msec at 0 mV and 750 msec at −10 mV. The currents shown in figure 5B were obtained by subtracting the current in 10^{-6} M from the current in the presence of 5×10^{-9} M; it represents the i_K current, which remains unblocked in the presence of 5×10^{-9} M. By calculating the ratio of the current in the presence of 5×10^{-9} M over the reference i_K current (C) at different times, it is possible to obtain the time course and extent of block. The result of such a procedure is shown in figure 5C. An initial value of 1.0 in this figure means no block. The example clearly shows that at 100 msec, a block already existed that was more prominent with greater depolarization. After this initial block, inhibition of the current slowly increased with time. Successful measurements were done in five experiments in which control, an intermediate (5×10^{-9}M) and a high concentration of the drug were applied. Values for the early block were 0.10 ± 0.05 at −20 mV, 0.27 ± 0.03 at −10 mV, 0.40 ± 0.02 at 0 mV and 0.53 ± 0.02 at +10 mV. Exact time constants for the increase in block during the rest of the pulse were difficult to estimate because of the pronounced noise level of the difference currents, but were in the order of seconds. These experiments, thus, show that the total block at the end of a depolarizing pulse consists of two components: an initial block, which we interpret as a fast open channel block (see later), followed by a rather slow second component. In the same experiments, tail currents and their ratios showed a block of 0.29 ± 0.03 at −20 mV, 0.40 ± 0.05 at −10 mV, 0.50 ± 0.04 at 0 mV and 0.56 ± 0.02 at +10 mV. Time constants for tail decay in the presence of the drug were not different from those in the control conditions. This last observation is of importance with respect to estimation of recovery from block.

Tail currents. The development of block can also be studied by measuring the change in tail current after clamp depolarizations of variable duration. Clamp pulses to 0 mV were applied for durations between 0.1 and 5 sec. An example of such an experiment is given in figure 6. Evolution of the tail current amplitude as a function of time in control conditions can be described by an exponential with a time constant of 352.7 ±

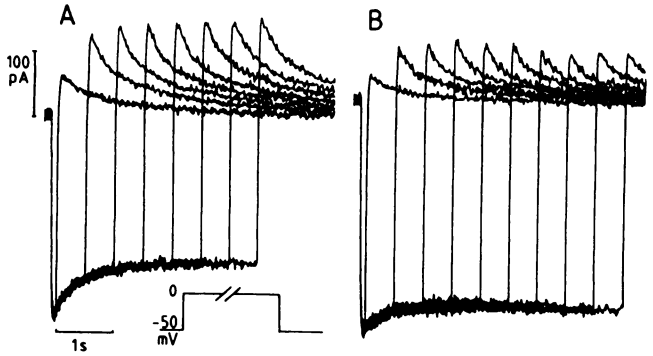

Fig. 6. Open-state block development estimated by tail current amplitude. The voltage clamp protocol consisted of clamp depolarizations from −50 to 0 mV for various durations. Currents during and after the depolarizations (tails) are shown for control conditions and in the presence of 5×10^{-9} M. Under control conditions (A), outward current during the clamp and tail amplitude increased to a plateau as a function of the duration. In the presence of the drug (B), both currents were decreased; instead of tending to a plateau, the currents decreased with time; rabbit ventricular myocyte.

9.45 msec (n = 6). From the example shown in figure 6A, it is also clear that the time course of the current during the depolarizing pulse is similar to the envelope of tail currents, indicating that a single i_{Kr} is present. In the presence of the drug (5×10^{-9} M), the evolution of the tail currents was complex and could not be described by a single exponential. Therefore, we analyzed the time course of block by plotting the ratios of tail currents. At the beginning (100 msec) of the conditioning pulses, this ratio was different from 1.0 in accord with the observation of rapid block upon depolarization. In nine cells, the early block determined in this way amounted to 0.22 ± 0.03. As the depolarizing pulse was prolonged, the ratio of currents further decreased. The time course could be quantitatively expressed by an exponential with time constant of between 4.4 ± 0.5 sec (n = 9) and an amplitude of 0.42 ± 0.02, leaving 0.36 ± 0.03 as the fraction of current not blocked in steady state.

Recovery from block. Different methods can be used to study recovery from block by a compound, depending on whether the time course of unblock is fast or slow. If recovery is fast, its time course can be studied by measuring the change in tail current time course. Channels that become unblocked during the tail will generate outward current and, thus, slow the decay of the tail. A detailed analysis of the tail current time course was not made, but by scaling the tails in the presence of dofetilide (not shown), it was found that their time course was not different from control. The results, then, suggest that unblock was not occurring during the decay of the tail.

A second method to study recovery from block is to apply a test depolarization given at variable times after the induction of block by a conditioning pulse protocol. The change in the tail of the test pulse is a measure of recovery. Figure 7 summarizes the results obtained from eight cells. The protocol consisted of three phases (a, b and c). Results of phases a and b have already been described in the sections on development of block at rest and use dependency. After development of the steady-state use-dependent block in phase b, the test voltage clamp was applied after either 1, 2 or 5 min. The holding potential was −50 or −75 mV. At −75 mV, no recovery was seen (*i.e.*, the tail currents, even after 5 min, remained at the low level obtained at the end of the series of voltage clamps). This result suggests that the drug does not leave the closed channel and seems to be trapped by the closed activation gate. At −50 mV, recovery was present, although slow (time constant order of min) and incomplete after 5 min.

Is block frequency dependent? Preferential inhibition of i_K at elevated frequencies is a characteristic that is considered advantageous to block tachyarrhythmias. In order to show such frequency dependency, the current should be blocked during depolarization (open channel block), and recovery from block should occur with a time constant in the order of the diastolic interval. Recovery at −75 mV, however, was shown to be very slow or nonexistent. From this observation, a constant steady-state block as a function of stimulus interval between 0.5 and 5 sec could be predicted. The results in figure 8 confirm this prediction. In seven preparations, depolarizing pulses of 200 msec up to +20 mV, followed by 100 msec at −50 mV (to measure the tail) and a return to −75 mV, were applied at a frequency variable between 0.2 and 2 Hz. It is clear that the relative decrease in tail amplitude was the same at all frequencies.

i_K in the Guinea Pig Ventricular Myocyte

Activation experiments similar to those described for rabbit ventricular myocytes were also performed on guinea pig ventricular myocytes. An example of the activation curve and its modification by dofetilide (3×10^{-8} M) is given in figure 9. In comparison with the data on rabbit cells, the following differences are noted: 1) under control conditions, the activation curve does not saturate at +10 mV, but continues to increase at more positive potentials; 2) the effect of the drug is more pronounced in the range of negative potentials, whereas relative

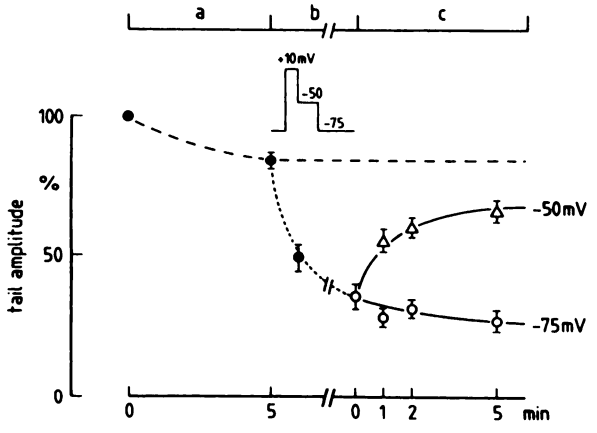

Fig. 7. Voltage-dependent recovery from block. Recovery was estimated by applying a test depolarization at variable times after the induction of block. Tail current amplitude was measured at −50 mV. Holding potential during recovery was either −75 or −50 mV. The total experiment consisted of three phases, a, b and c. In a first phase (a) after measuring the control tail amplitude for the test pulse (see inset), the drug (3×10^{-9} M) was washed in during 5 min, while the membrane was held at −75 mV. After 5 min, the test pulse was applied and the tail was measured. The test pulse was then repeated at a frequency of 1 Hz (phase b) until the tail amplitude declined to a steady state; measurements after 1 min and in steady state are given. During a third phase (c), recovery was measured at 1, 2 and 5 min. Mean values ± S.E. for eight rabbit ventricular myocytes.

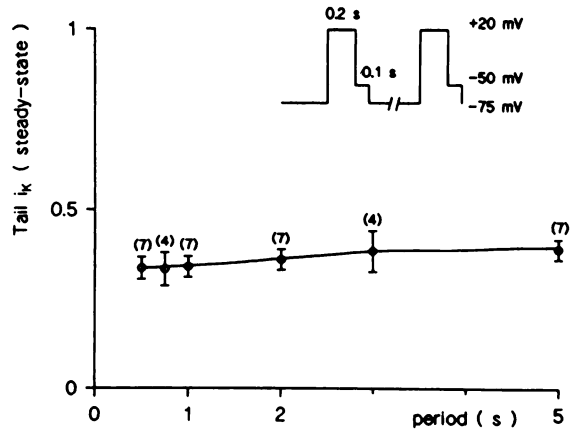

Fig. 8. Absence of steady-state frequency-dependent block. The voltage clamp protocol shown in the inset was repeated, with intervals shown on the abscissa. Tail currents were measured at −50 mV. The ordinate gives the ratio of the tail in the presence of 5×10^{-9} M of the drug over the tail in control conditions. The number of cells is in parentheses; rabbit ventricular myocytes.

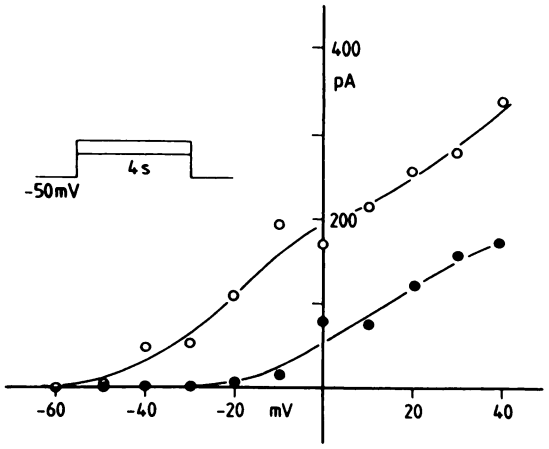

Fig. 9. Activation curve and effect of 3×10^{-8} M dofetilide in a guinea pig ventricular myocyte. Voltage steps to different levels from a holding potential of −50 mV. Tail currents are given on the ordinate. In comparison with the data in rabbit myocytes (fig. 2), tail currents in the guinea pig do not saturate at positive potentials. In the presence of the drug, tails are suppressed at moderate depolarizations, but less at positive potentials, show a voltage dependency opposite to that in figure 2.

block decreases with more positive test potentials. This kind of voltage-dependent effect seems to be opposite to the results described in the rabbit.

The experiments described below demonstrate that the divergent effects in the guinea pig can be explained by the existence of two components of the delayed K^+ current, with different activation voltage ranges and different sensitivities to the drug. The existence of two components with different kinetics, i_{Kr}, a rapidly developing current for moderate depolarizations, and i_{Ks}, a slowly developing current for large depolarizations, has recently been proposed by Sanguinetti and Jurkiewicz (1990); these authors also mentioned the low sensitivity of i_{Ks} to the experimental drug E 4031. The present experiments have confirmed the existence of these two components and their difference in sensitivity. The experiment in figure 10A shows that when the repolarization step from +30 mV is subdivided in a step to −10 mV followed by a second step to −50 mV, the drug, in a concentration of 10^{-8} M, had no effect on the tail at −10 mV but completely inhibited the tail at −50 mV. The experiment suggests that the i_K channels deactivated at −10 mV are insensitive, whereas the channels deactivated at −50 mV are very sensitive to blockade. Based on these findings, we have studied the effect of dofetilide on the activation curve obtained either from a holding potential of −50 mV (fig. 10B) or a holding potential of −20 mV (fig. 10C). As is evident, the tails at −20 mV were insensitive to 3×10^{-8} M of the drug, whereas the tails at −50 mV were completely blocked. Because the tails for both components were measured at different potentials, it could be argued that the difference in block is due to the drug being more effective at −50 mV than at −20 mV (a situation opposite to that in the rabbit). This explanation, however, can be ruled out by the results reported in figure 9. In this latter case, all tails were measured at the same potential of −50 mV; the tails for moderate depolarizations were completely blocked (i_{Kr}), whereas the tails after large depolarizations ($i_{Ks} + i_{Kr}$) were only reduced to half their control value.

Absence of Effect of UK on i_{KI}, i_{to} and i_{Cl}

The inward rectifier (i_{KI}). The inward rectifier in heart is responsible for the negative resting potential, and carries large

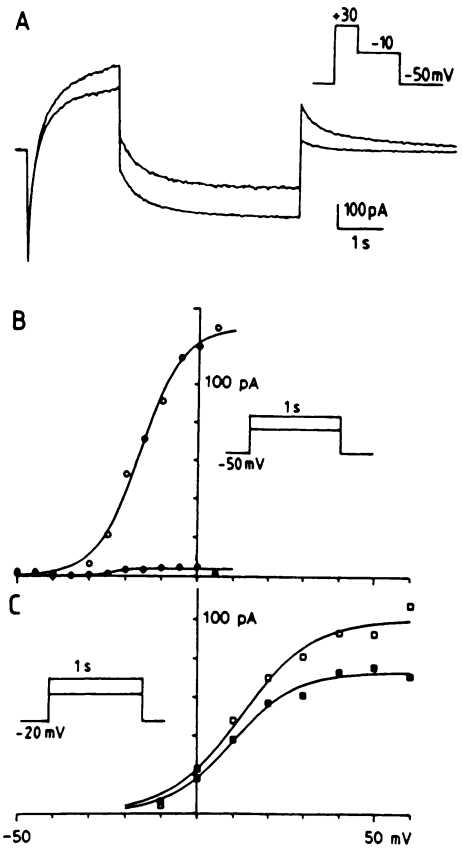

Fig. 10. Existence of two i_K components with different sensitivity to block in the guinea pig ventricular myocyte. A) Deactivation at two different potentials, −10 and −50 mV. In the presence of 10^{-8} M, the current which deactivates at −10 mV is insensitive, whereas the current which deactivates at −50 mV is practically suppressed. B and C) Activation and deactivation at two different potentials. Clamp depolarizations were applied from a holding potential of −50 or −20 mV. The current, which is activated and deactivated in the voltage range of −50 to 0 mV, is very sensitivity to block by 3×10^{-8} M of the drug, whereas the current in the voltage range positive to 0 mV is practically not changed.

inward currents, which inactivate incompletely at potentials negative to the equilibrium potential; the outward current component of the current-voltage relation, which is of physiological importance, shows a prominent negative slope for potentials higher than −70 mV. The effect of dofetilide on outward and inward currents through the i_{KI} channel was tested in three rabbit ventricular myocytes. The results illustrated in figure 11 show that large concentrations of the drug neither affected the outward currents (3×10^{-8} M) nor the instantaneous and steady-state inward currents (10^{-5} M).

i_{to}. The i_{to} current was studied in rabbit Purkinje cells and rat ventricular myocytes. In both preparations, this type of channel is well expressed. An example of a recording in a rabbit Purkinje cell is given in figure 12 and illustrates a pronounced i_{to} during a voltage step from −50 to 0 mV, compared to a small i_K tail on repolarization to −60 mV (for a more detailed analysis of i_K in single rabbit Purkinje cells, with or without i_{to} present, see Scamps and Carmeliet, 1989). In the presence of 3×10^{-8} M, the i_{to} current was not affected, whereas the i_K tail was completely inhibited. Similar effects were seen in four other Purkinje cells. Two experiments were performed in rat ventricular myocytes. In this preparation, the i_{to} current is the major contributor to the outward current during depolarizing steps, whereas i_K is negligible. A concentration of 10^{-6} M dofetilide

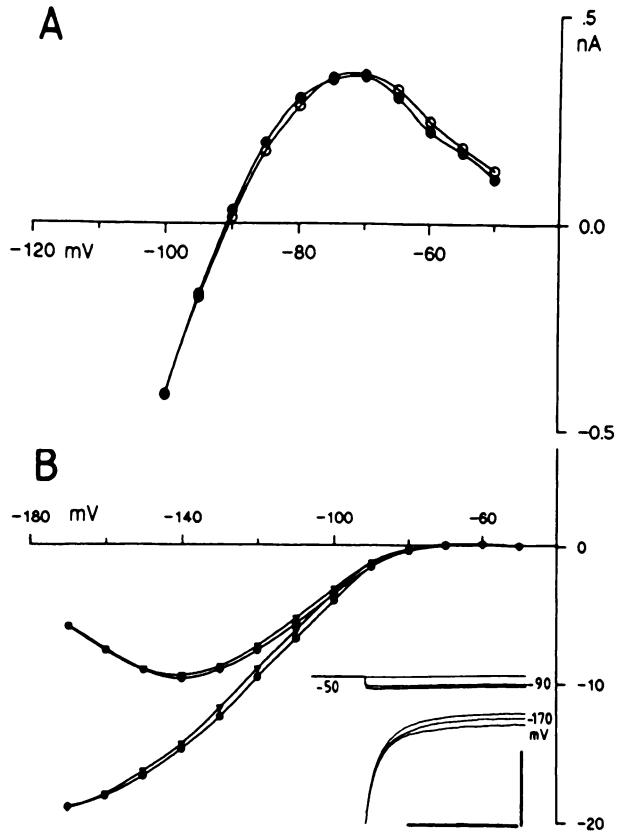

Fig. 11. Absence of effect of dofetilide on the inward rectifier, i_{Ki}. A) Step clamps were applied negative and positive to the resting potential and show the absence of effect of the drug (3×10^{-8} M) (●) on the inward rectifying part of the current. B) Hyperpolarizing steps up to −170 mV were applied to show the part of i_{Ki}, in which the current is inactivated. Even at 10^{-6} M (▲), the drug was without effect. The inset illustrates currents for clamp to −80 and −170 mV; callibration, 100 msec and 10 nA.

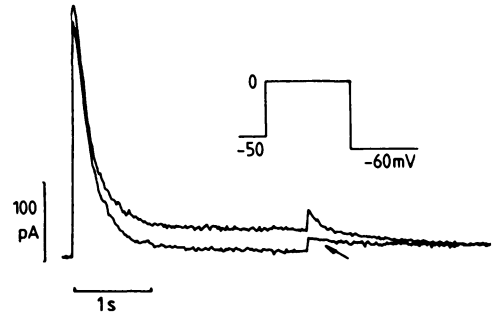

Fig. 12. Absence of effect of dofetilide (3×10^{-8} M) on the transient outward current in a single Purkinje cell; under the same condition, the i_K current is blocked (arrow). See inset for voltage clamp protocol.

had no effect on the outward current for a step to +20 mV from a holding potential of −80 mV. The absence of an effect was verified for voltage steps between −40 and +40 mV (results not illustrated).

i_{Cl}. On β-receptor stimulation, the cardiac action potential is shortened. This result is due to enhancement of i_K, but in certain species, it is a result of activation of a i_{Cl}. In order to predict the effect of a drug on the action potential duration under those conditions, it is of interest to know its effect on i_{Cl}.

i_{Cl} was studied in rabbit and guinea pig ventricular myocytes. For the rabbit preparation, the method was similar to that used by Harvey and Hume (1989). To reduce i_K activation as much as possible, internal and external solutions (see "Methods") contained Cs^+ instead of K^+, temperature was lowered to 23°C and only short pulses (100 msec) were applied from a holding potential of −30 mV. The results are shown in figure 13 for control conditions (A), presence of isoproterenol (10^{-6} M) (B) and isoproterenol plus dofetilide (10^{-7} M) (C). Addition of isoproterenol induced a current with a reversal potential of −22 mV (see difference current in D) and a current-voltage relationship which was outwardly rectifying. This reversal potential is close to the theoretical E_{Cl} of −30 mV. The addition of dofetilide (10^{-7} M) did not affect the isoproterenol-induced current. The same negative result was obtained in a second rabbit myocyte.

Two other experiments performed on guinea pig ventricular cells confirmed these results. In these experiments, standard intracellular solution was used; K^+ currents were blocked by addition of 1 mM Ba^{++} to the external solution, Ca^{++} currents were eliminated by nisoldipine (10^{-6} M) and temperature was 37°C. Addition of 10^{-7} or 10^{-6} M dofetilide had no effect on the time-independent current elicited during depolarizing clamps.

Discussion

In the present analysis, the effects of dofetilide on the two components of the delayed K^+ current, i_{Kr} and i_{Ks}, have been described. The two components can be distinguished by their activation kinetics, rectification and drug sensitivity (Sangui-

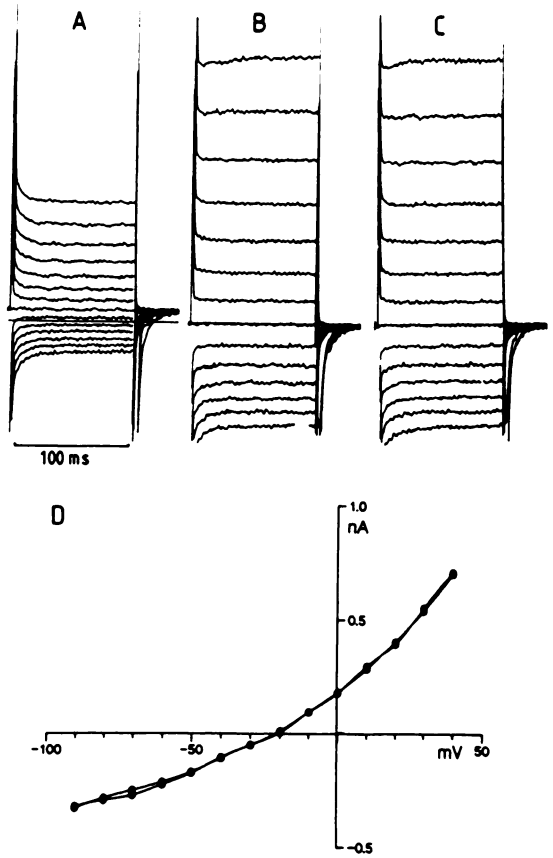

Fig. 13. Absence of effect of dofetilide on the isoproterenol-induced i_{Cl}. A–C) Examples of currents under control conditions (A), in the presence of isoproterenol (10^{-6} M) (B) and in the presence of isoproterenol plus dofetilide (10^{-7} M) (C). In D, the i_{Cl} is obtained from the difference in currents, B − A and C − A. At 10^{-7} M, the drug had no effect on the Cl^- current.

netti and Jurkiewicz, 1990; Colatsky *et al.*, 1990). I_{Kr} is rapidly activated (0.1–0.5 sec), and for moderate depolarizations, between −40 and 0 mV; the fully activated current-voltage relationship shows inward rectification, and the current is blocked by E-4031. I_{Ks}, on the other hand, is slowly activated, and only for depolarizations positive to −10 mV with no saturation up to +50 mV; rectification is absent or minimal and the current is insensitive to E-4031.

The present results have shown that dofetilide blocks selectively i_{Kr}, the rapidly developing component of the delayed K^+ current. Other currents such as i_{Kl}, i_{Cl} or i_{to} were not affected. The block of i_{Kr} was highly efficient, with a K_D of 3.9×10^{-9} M. It was also more prominent at positive potentials and developed during depolarization in two phases, a very rapid and a rather slow one. Recovery was very slow at hyperpolarized levels. Although a small component of use dependency could be experimentally demonstrated, steady-state block as a function of frequency did not vary.

Depending on the species, the delayed K^+ current exists of a rapidly activated i_{Kr} component followed by a slowly activated i_{Ks} component, or only of the rapidly activated component (Colatsky *et al.*, 1990). The two components are present in guinea pig atrial and ventricular myocytes, sheep Purkinje fibers and chick atrial muscle. In rabbit preparations (nodal, Purkinje and ventricular cells) and cat ventricular muscle, only the rapid component is seen. Selective blockade of the i_{Kr} has been shown for E 4031 and sotalol. The present comparative analysis in rabbit and guinea pig demonstrates that dofetilide can be considered a selective blocker of i_{Kr}, and furthermore, that it blocks this component in a voltage-dependent way. The more pronounced block at depolarized levels is consistent with the hypothesis that the positively charged drug has access to the open channel from the intracellular side. The preferential blockade of i_{Kr} is also in accord with observations by Gwilt *et al.* (1991), who remarked that even at the high concentration of 2×10^{-6} M dofetilide, tail currents could still be observed in response to large voltage steps.

During a depolarizing voltage step, block of i_K increased with a time constant in the order of seconds. Extrapolation to zero time indicated the existence of a fast developing open state block or a preexisting rested state block. The fact that this "instantaneous" block was voltage dependent seems to rule out the hypothesis of rested state block. Absence of block of channels in the rested state was confirmed in experiments in which the membrane was held at −75 mV during 5 min; on applying a test depolarization, the small decrease in tail was far below the value expected in steady state. We therefore conclude that depolarization by dofetilide is an open state block and occurs in two phases, a very fast and a slow phase.

Open state block has also been demonstrated for quinidine in rabbit sinoatrial and atrioventricular nodes (Furukawa *et al.*, 1989). The delayed K^+ current in these cells is of the i_{Kr} type (see also Nakayama *et al.*, 1984 and Shibasaki, 1987). In an analysis on guinea pig myocytes, Balser *et al.* (1991) came to the opposite conclusion and claimed block at rest and unblocking during depolarization. However, if two components of i_K with different sensitivities are considered, their data are not in contrast with the interpretation of an open state block of the i_{Kr} component.

Recovery from block by dofetilide occurred very slowly; no fast phase could be distinguished. It was, furthermore, voltage dependent, as recovery was extremely slow at hyperpolarized levels. This finding suggests that unblock does not occur from the rested state. On hyperpolarization the drug seems to be trapped by the rapid closure of the activation gate (fast deactivation). In terms of a possible state dependence block model, this means 1) that block and also unblock occur *via* the open state, 2) that transition between the blocked open state and blocked rested state is possible and 3) that block or unblock does not occur *via* the rested state. Information on recovery from block by other drugs acting on i_K is presently unavailable.

From the existence of a fast and slow phase in the development of block together with a slow recovery from block, it is possible to predict existence of use-dependent block. Use dependency was obtained when long (>1 min) resting periods were interpolated between series of voltage clamp depolarizations. However, when clamp depolarizations of 200 msec duration, comparable to the normal duration of the action potential, were repeated at intervals between 0.5 and 5 sec, no change in relative block of tail current could be observed. In other words, steady-state block as a function of frequency was constant or appeared as a tonic block. This is understandable because recovery time constants during the hyperpolarized state are on the order of minutes and, thus, much longer than the time interval between depolarizations. At all frequencies, this resulted in the same steady state, which is only dependent on the concentration of the drug.

Because block of i_K with frequency is constant, one does not expect any large difference in relative action potential lengthening with frequency. Such a result was obtained by Tande *et al.* (1990) in guinea pig at 10^{-8} M; at higher concentrations, the relative lengthening was less pronounced at high frequencies. A similar observation was made by Gwilt *et al.* (1991), again at high concentrations of 10^{-7} to 10^{-6} M. Such an effect, however, should not be called reverse use dependency (Hondeghem and Snyders, 1990). Use dependency is a notion which refers to interaction of a drug with the channel. "Normal" use dependency means that the block increases when the channel is activated, and eventually inactivated. We have demonstrated that the drug exerts such normal use dependency. The absence of a steady-state frequency-dependent block is not contradictory with this statement, and is due to the time constant of recovery, as it is much longer than the time between depolarization when frequency is changed. When extrapolation is made to the action potential duration, changes of or interaction with other currents should be taken into account. It should be stressed, furthermore, that results in the guinea pig (with i_{Kr} and i_{Ks}) will be different from those in other species, such as the rabbit or the cat, where only i_{Kr} is present. It would, thus, be worthwhile to study the frequency-dependent effect of dofetilide on the action potential duration in the rabbit and the cat and to compare it with the results in the guinea pig. Extrapolation to the human species is not possible at the present time because no detailed information is available about the kinetics of the delayed K^+ current in this species.

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