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Use-Dependent Block of the Delayed K^+ Current in Rabbit Ventricular Myocytes

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Summary. **Block of the delayed K^+ current, i_K, and the concomitant increase in refractoriness is considered an alternative to a decrease of conduction in the treatment of reentry arrhythmias. Ideally an agent should selectively prolong the action potential at high frequencies. A minimum requirement is use-dependent block. A number of drugs were tested for the existence of use dependence by applying a train of depolarizing clamps to single cardiac myocytes of the rabbit ventricle. Development of block during a long depolarizing clamp and recovery from block were also measured. Five of the nine drugs tested, i.e., disopyramide, encainide, quinidine, sotalol, and tedisamil, did not show use dependence. When a train of depolarizing clamps was applied, block was already present for the first depolarization and did not increase with repetition of the pulse. This result suggests block of the channel in the rested state or a very fast block of the open channel. Almokalant and amiodarone, and to a lesser extent dofetilide and E4031, showed use-dependent block, i.e., block increased during the train of depolarizing clamps. The time constant for the open channel block was 1.07 seconds for almokalant and 0.67 seconds for amiodarone. Recovery from block for almokalant and amiodarone was very slow: time constants measured at −50 mV were 13.9 and 12.7 seconds, respectively. For dofetilide it was in the order of minutes. The existence of this slow recovery explains why frequency-dependent changes in block were negligible or absent for frequencies above 0.5 Hz. Future research should be aimed to select drugs with a slower onset of active state block and faster recovery from block.**

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Key Words. **K+ current, open channel block, use-dependent block, action potential duration, action potential frequency**

Since the CAST study [1] has emphasized the inherent arrhythmogenicity of drugs that reduce conduction velocity (class I drugs), attention has been shifted recently to drugs that prolong the action potential (class III drugs). Instead of silencing the activity in the reentry pathway by decreasing conduction velocity, the same effect can be obtained by increasing the action potential duration or the refractory period, the result being in both cases a prolongation of the wavelength. For an agent with class III activity, restriction of this activity to elevated frequencies, with no effect on the action potential at low frequencies, represents certain advantages [2]. Prolongation of the action potential at low frequencies may indeed be arrhythmogenic and lead to arrhythmias of the torsades de pointes type [3,4]. A minimum but not necessarily sufficient requirement for selectivity at high frequencies is the existence of use dependence. Demonstration of use dependence furthermore provides interesting information on the mechanism of blockade.

It has been suggested that the marked prolongation of the action potential at low frequencies by quinidine is due to the existence of reverse use dependence [2]. In the present communication (see also Carmeliet [5]), we will show, however, that normal use dependence can be demonstrated for amiodarone and almokalant, and to a certain extent also for dofetilide and E4031, while others, such as quinidine, solatol, encainide, tedisamil, and disopyramide, only show tonic block. In no case were we able to demonstrate reverse use dependence. The present results call for a clear distinction between steady-state frequency-dependent effects on action potential duration and use-dependent block at the channel level. The data described in this report are original, except for dofetilide [8].

Methods

Single ventricular myocytes of the rabbit were dissociated by enzymatic dispersion, following a procedure described in detail in a previous publication [6]. In brief, the heart was quickly removed after decerebration of the animal and was mounted on a Langendorf perfusion system. The aorta was cannulated and the heart was perfused at 37°C with (a) Ca^{2+}-free standard solution (see solutions) for 10 minutes; (b) Ca^{2+}-free standard solution containing 35 mg/50 ml collagenase A (Boehringer Mannheim) for 15 minutes; (c) a Ca^{2+}-free solution containing collagenase and 6.5 mg/50 ml protease XIV (Sigma, St. Louis, MO) for 35 minutes, and (d) 0.2 mM Ca^{2+}-containing solution for

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an extra 10 minutes. Following the isolation of cells, they were stored at room temperature in Hepes-Tyrode until used.

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, Burlingame, CA) was applied using suction pipettes with resistance ranging from 2 to 5 MΩ. P-clamp software (Axon Instruments, Burlingame, CA) 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. Results are expressed as mean values ± standard error.

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. The intracellular solution contained in mM: KCl 120, $MgCl_2$ 6, $CaCl_2$ 0.154, Na_2 ATP 5, EGTA 5, and HEPES 10, with KOH added until pH 7.2. The following drugs were used: almokalant (Astra Hässle, Sweden), amiodarone (Labaz, Belgium), disopyramide (Sigma, St. Louis, MO), dofetilide (Pfizer, England), E4031 (Esai, Japan), encainide (Bristol Myers, Belgium), quinidine (Sigma, Belgium), sotalol (Bristol Myers, Belgium), and tedisamil (Solvay Pharma, Germany).

Results

Different types of use dependence

In order to study use-dependent block a train of short (200 msec) depolarizing clamps to 0 or +10 mV from a holding potential of −50 mV were applied to a rabbit myocyte at a repetition frequency of 1.33 Hz. Figure 1 shows the change in tail current during the train of depolarizing clamps under control conditions and in the presence of 10^{-6} M of amiodarone after 5 and 10 minutes of superfusion. Under control conditions the tail current rapidly increased to a saturating value with successive depolarizations. 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 the first tail was hardly different from the control, indicating that the drug did not bind to the channel in the rested state; with repetition of the pulse, however, the tail amplitude instead of showing an increase steadily decreased. This effect was more pronounced after a longer time of superfusion. Since most of the i_K channels may be assumed to be in the rested state at the holding potential and change to the open or activated state during the depolarization, the results demonstrate blockade of open channels and incomplete recovery during the rest period between pulses.

Use-dependent block was quantified by calculating the ratios of the tail current in the presence and absence of the drug. The time course of change in the ratios was fitted by an exponential. For amiodarone 10^{-6} M and the clamp protocol used, this time constant in terms of number of pulses was 3 to 4 (n = 4; see also Fig. 2).

Similar results were obtained with almokalant [7]. At a concentration of 5×10^{-8} M and after sufficient time at the holding potential of −50 mV, the tail current was hardly changed at the start of the series of depolarizing clamps; in other words, there was no tonic block. With repetition of the clamps the tail current gradually decreased. This indicates that block develops during activation, does not recover completely during the rest period at the holding potential, and thus accumulates with time. The time constant for the ratio of tail currents was again on the order of a few pulses.

Results obtained with other drugs known to inhibit the delayed K current were different. In one group that can be considered the other extreme, no use-dependent block was seen: The amplitude of the tail current was already reduced from the first applied depolarization and did not change with repetition of the clamp. Such a result may be called *tonic blockade.* This type of behavior was seen with the following drugs: quinidine, sotalol, disopyramide, tedisamil, and encainide. An example for quinidine is given in Figure 2. Two possible explanations can be proposed: The drug either blocks the channel already at the rested state, i.e., at the holding potential of −50 mV, or block develops so fast during the depolarization that a steady state is obtained during the first pulse. In this respect the duration of the pulse and the concentration of the drug are of critical importance. If the drug is a fast open-channel blocker, shorter pulses and lower concentrations may eventually reveal use dependence for these drugs. The actual protocol, however, was selected to allow for an easier interpretation of the data in relation to block during normal electrical activity. Although the clamp does not model the action potential in a rigorous way, the amplitude and the duration are such that the results provide useful information for changes occurring during an action potential.

In a third group, results were intermediate and block consisted of a tonic and phasic component. This was the case for E4031 and dofetilide (see Carmeliet [8] for a complete description) and an example is given in Figure 2.

Block development and recovery from block

The existence of use-dependent block for a current that only undergoes activation and deactivation, means block of open channels during the depolarizing pulse and incomplete recovery from block during the time at the holding potential. For amiodarone, dofetilide, and almokalant, we have measured the development of block during a single depolarization as well as the recovery from block at −50 mV. The development of block was measured by the change, i.e., decrease in

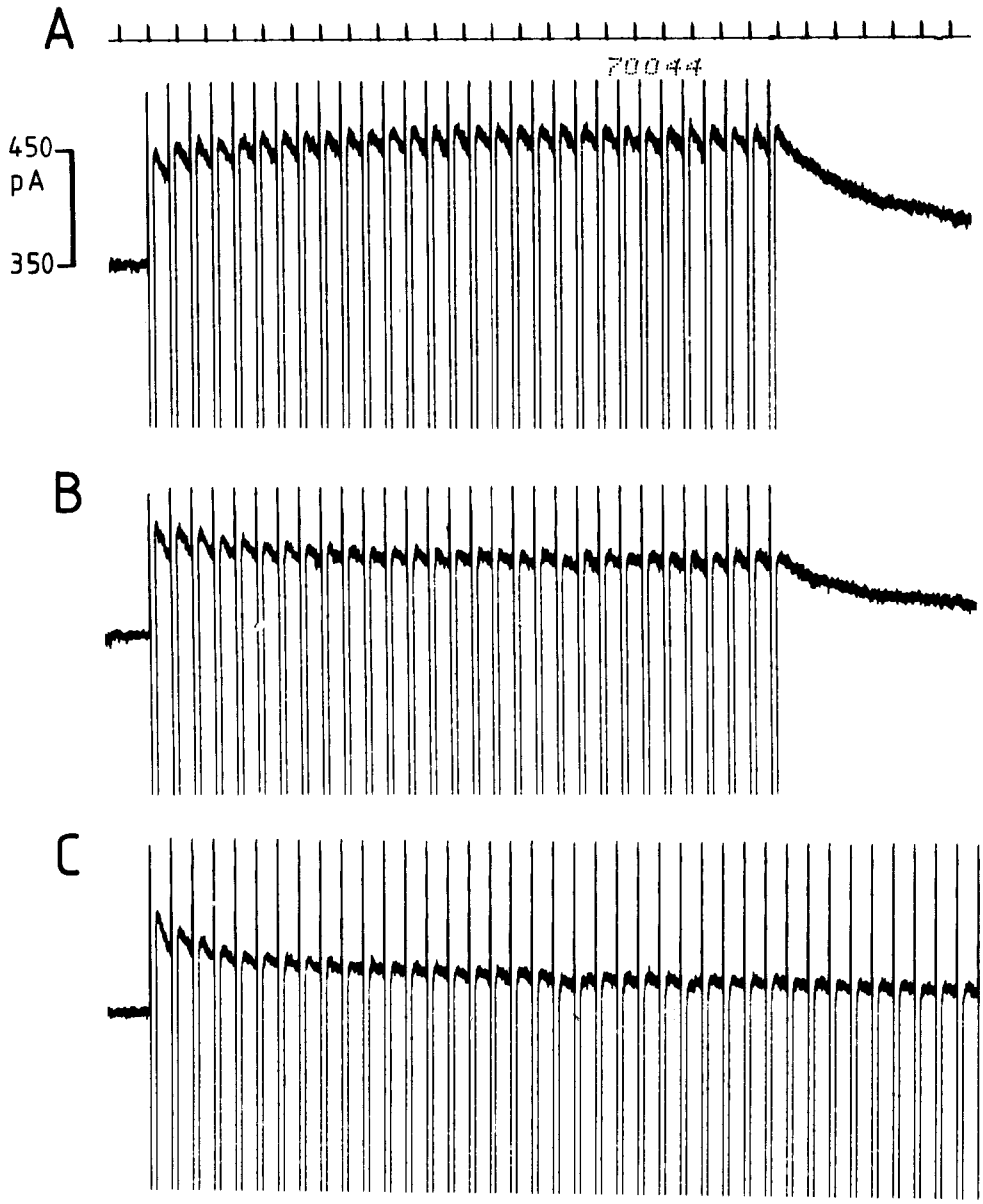

Fig. 1. *Use-dependent block of the delayed K^+ current by amiodarone. Change in tail current during a train of depolarizing clamps under control conditions (A) and in the presence of 10^{-6} M amiodarone after 5 (B) and 10 (C) minutes of superfusion. Following a rest period of 2 minutes at the holding potential of −50 mV, short (200 msec) depolarizing clamps to 0 mV were applied to a rabbit monocyte at a repetition frequency of 1.33 Hz. Tail current is measured on return to −50 mV. Under control conditions the tail current, which is a measure of the delayed K^+ current, rapidly increased to a saturating value with successive depolarizations (A). In the presence of the drug (5 minutes) tail currents showed the opposite behavior and gradually decreased (B). This negative staircase effect was more pronounced after a longer time of superfusion (10 minutes; C). Rabbit ventricular monocyte at 37°C. The upper line indicates 1 second intervals.*

tail current after clamp durations of variable duration (0.1–5 seconds). Recovery from block was studied by first inducing steady-state block by a series of clamp steps and then applying a test depolarization at variable intervals to measure the tail current. For amiodarone 5×10^{-7} M, block developed at +20 mV with a time constant of 0.67 ± 0.14 seconds (n = 9), and recovery at −50 mV occurred with a time constant of 12.7 ± 1.4 seconds (n = 8). For almokalant 5×10^{-8} M, these values were 1.07 seconds (n = 4) and 13.9 seconds (n = 3; time constant of the exponential drawn through all experimental values combined). For dofetilide 5×10^{-9} M, block development consisted of two phases: an initial very fast within a few

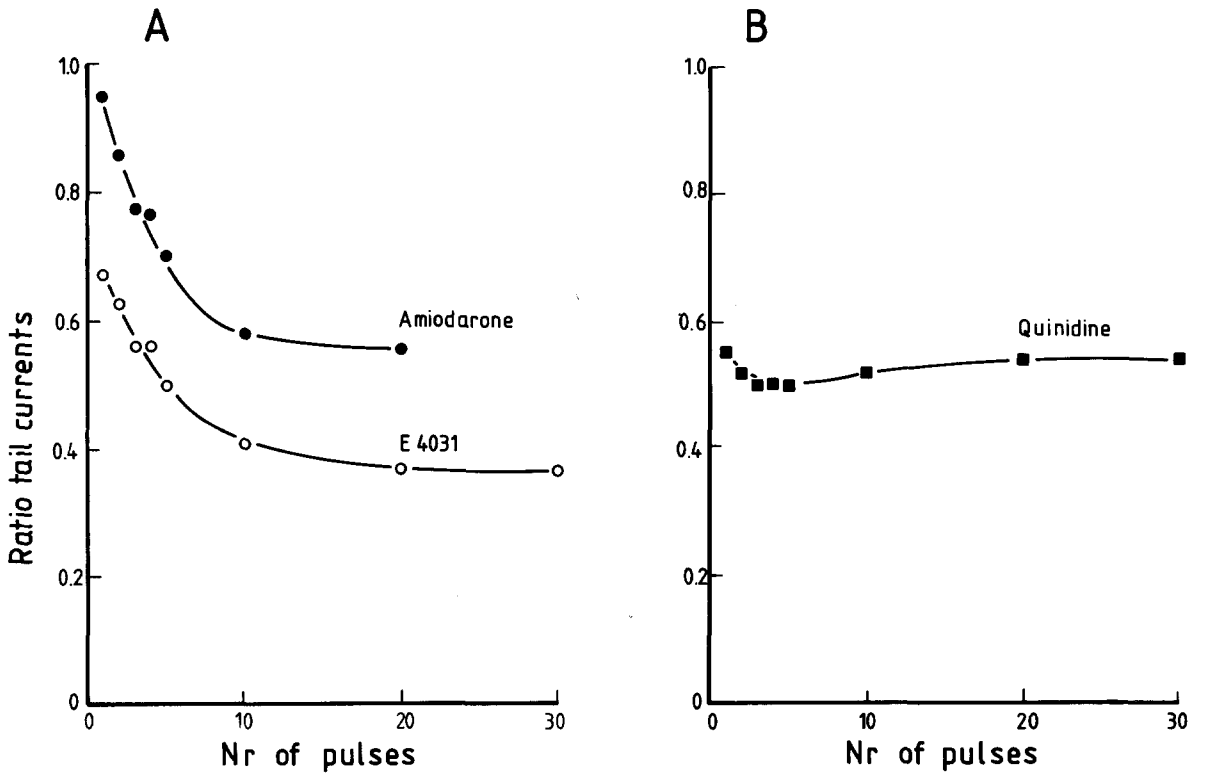

Fig. 2. Different types of use-dependent block. Ratio of tail currents in the presence and absence of drug as a function of number of depolarizing pulses applied. The ratio is a measure of the i_K current remaining unblocked. The voltage clamp protocol was the same as in Figure 1. Values are given for the five initial pulses, and the 10th, 20th, and 30th pulses. The type of drug is given in the figure. With amiodarone 10^{-6} M (A) the i_K current was hardly or not affected for the first pulse (see also Figure 1B) but developed with time until steady state was reached between the 10th and the 20th depolarizations. With E4031, 3×10^{-9} M (A), a block was already apparent at the end of the first pulse; this block was followed by a second use-dependent increase in block, until steady state was obtained around the 20th pulse. With quinidine (6×10^{-7} M; B) block was already pronounced for the first pulse but did not change with repetition. There is only a tonic block. The block present at the end of the first pulse with E4031 and quinidine indicates a block of the channel in the rested state or a very rapidly developing block during the 200 msec of the first pulse. Rabbit ventricular myocyte at 37°C.

milliseconds followed by a very slow phase with time constant of 4.4 ± 0.5 seconds (n = 7); recovery in this case was extremely slow with a time constant on the order of minutes [8].

Discussion

Frequency-dependent block of i_K

Drugs that showed use dependence in the test applied in this study will not necessarily show a frequency dependence in the physiologic range of heart rates. Existence of a frequency dependence at rates above 0.5 Hz requires that recovery is sufficient during diastole, i.e., the time constants should be of the order of the duration of diastole. For amiodarone and almokalant, and even more so for dofetilide, the time constant of recovery was much longer (order of 10 seconds to minutes). These drugs therefore will show use dependence only for a few seconds after their initial application to the tissue. Block will gradually increase during these initial activations and attain a steady state. Due to their long dissociation time constant, essentially no unblocking occurs during diastole, and therefore the steady-state block will be the same and independent of frequency. In the case of dofetilide, experiments have shown that block remained constant for steady-state stimulation at intervals between 5 and 0.5 seconds [8]. Extrapolating from current to action potential duration, the situation is even worse. Indeed it is well known that frequency itself modulates the action potential and that its duration is longer at low frequencies. Since the time constant for development of block is in the order of 1 second or smaller, the drug will block i_K substantially during the course of the long action potential at low frequencies. Although a drug may show normal use dependence, the expected frequency dependence may thus not occur for two reasons: the very slow recovery from block at the resting potential and the relative short time constant for block development.

Normal and reverse use dependence

The analysis in the present report has shown (a) that drugs such as almokalant, amiodarone, and dofetilide block the i_K channel during the depolarizing pulse, i.e., in the open state, (b) that block increases during a train of depolarizations (use-dependent block), but (c) do not show the expected frequency-dependent block and thus will not cause the selective prolongation of the action potential at high frequencies. A drug can show normal use dependence but not the expected frequency dependence. It is therefore clear that the absence of a selective effect at high frequencies should not be called *reverse use dependence*. For the description of steady-state frequency effects also the term *use dependence* should be avoided.

It may further be stressed that in the present experiments no drug showed reverse use dependence. Unblocking during application of a train of depolarizing pulses did not occur. The conclusion of Hondeghem and Snyders [2] in favor of reverse use dependence in the case of quinidine was based on experimental results obtained in the guinea pig. Recent results allow another interpretation when the complex nature of i_K is taken into account. In the guinea pig ventricle, the total delayed K^+ current is indeed composed of two components: i_{K2}, which is rapidly activated at small depolarizations, and i_{Ks}, which is slowly activated and only for large depolarizations [9,10]. They further differ in their rectification characteristics and sensitivity to drugs: i_{Kr} is easily blocked by E4031 [9] and dofetilide [8], while i_{Ks} is more resistant. The results obtained by Roden et al. [11] and Balser et al. [12], such as the greater block during the initial phase of a large and long depolarization, as well as the greater block for small depolarizations to rather negative potentials, were interpreted as unblocking during the open state. However, they can also be explained by a selective block of i_{Kr} in the open or activated state. In support of this explanation open or activated channel block by quinidine has been demonstrated for the i_{Kr} channel in cardiac nodal tissues [13] and for a cloned human cardiac HK2 channel [14]. Time constants for block development were on the order of a few to tens of milliseconds. Demonstration of open state block is opposite to and excludes reverse use dependence.

Summary and Conclusions

1. A number of drugs blocking the delayed K current were tested for the existence of use dependence. When a train of depolarizing clamps was applied, block was already present for the first depolarization and did not increase with repetition of the pulse for the following drugs: disopyramide, encainide, quinidine, sotalol, and tedisamil. This result suggests block of the channel in the rested state or a very fast block of the open state.
2. Almokalant and amiodarone, and to a lesser extent dofetilide and E4031, showed use-dependent block, i.e., block increased during the train of depolarizing clamps. The time constant for open channel block was 1.07 seconds for almokalant and 0.67 seconds for amiodarone.
3. Recovery from block for almokalant and amiodarone was very slow: Time constants measured at −50 mV were 13.9 and 12.7 seconds, respectively. For dofetilide it was on the order of minutes. The existence of this slow recovery explains why frequency-dependent changes in block were negligible or not existent for frequencies above 0.5 Hz.
4. Future research should be aimed to select drugs with a slower onset of active state block and faster recovery from block.

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