British Journal of Pharmacology (1996) 117, 1427–1434

Increase in action potential duration and inhibition of the delayed rectifier outward current I_K by berberine in cat ventricular myocytes

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1 In the present work, the effects of the antiarrhythmic drug, berberine, on action potential and ionic currents of cat ventricular myocytes were studied.

2 Berberine prolonged action potential duration in cat ventricular myocytes without altering other variables of the action potential.

3 The drug at concentrations of 0.3–30 μM blocked only the delayed rectifier (I_K) current with an $IC_{50}=4.1$ μM. Berberine produced a tonic block and a phasic block that was increased with the duration of the depolarizing pulse. The blocking effect on I_K was use-dependent, but not frequency-dependent.

4 In cardiac preparations two delayed rectifier currents have been found: a rapid (I_{Kr}) current and a slow (I_{Ks}) current. In the present work it has been found that berberine at the concentrations used, selectively blocked I_{Kr}.

5 At concentrations higher than 10 μM it also decreased the transient outward (I_{to1}) current. The drug did not have effects on the inward rectifier (I_{K1}) or the high threshold calcium current ($I_{Ca\text{-}L}$).

6 These results show that berberine is a specific potassium channel blocker. The increase in action potential duration induced by berberine can be explained mainly by its blocking effects on I_K.

Keywords: Berberine; cardiac myocytes; action potential; ionic currents

Introduction

It has been shown that arrhythmias such as ventricular fibrillation can be the cause of haemodynamic collapse and cardiac sudden death, occurring in patients suffering from ischaemic heart disease or congestive heart failure (Lown, 1979; Bigger *et al.*, 1984; Packer, 1987; Olshausen *et al.*, 1991). The results of the Cardiac Arrhythmia Suppression Trial (CAST) have shown that blockers of the fast Na^+ channel (class I), flecainide and ecainide, although very effective in suppressing premature ventricular depolarizations, increase mortality in patients with recent myocardial infarction (Echt *et al.*, 1991). Class III agents that prolong action potential duration without depressing conduction might provide a valid alternative (Sicilian gambit). The antiarrhythmic benefit afforded by class III agents is proposed to result from sufficient prolongation of myocardial refractoriness for the wavelength of activation to exceed the path length of the re-entrant circuit, thereby preventing the initiation or maintenance of re-entrant excitation (Jansen, 1986; Frame & Bernstein, 1986).

In guinea-pig cardiac atrial and ventricular myocytes two different delayed rectifier outward currents have been found, a rapid one or I_{Kr}, sensitive to E-4031 and dofetilide, and a slow one I_{Ks} (Sanguinetti & Jurkiewicz, 1990). In cat ventricular myocytes using the 'envelope of tails test', the delayed rectifier current has been found to be composed of a single component (Furukawa *et al.*, 1992). In addition, Follmer & Colatsky (1990) have found that I_K in cat ventricular myocytes is completely blocked by E-4031, suggesting that only I_{Ks} is present in this preparation.

Berberine is a benzodioxoloquinolozine alkaloid occurring in plants of the genera *Berberis* and *Coptis*. It has been shown that berberine has a protective effect on cardiac arrhythmias induced by postischaemic reperfusion and other factors (Krol *et al.*, 1982; Kwiezycka *et al.*, 1983). Berberine prolongs APD and ERP in Purkinje fibres and ventricular muscle (Ricciopo Neto, 1993). In the present work the effects of berberine on action potential and the underlying ionic currents in cat ventricular myocytes have been studied. Berberine was found to increase action potential duration and this effect can be explained by a blocking effect on I_K. In addition, the possibility was explored of use-dependent and rate-dependent effects. Demonstration of use-dependence can provide interesting information on the mechanism of blockade.

Methods

Single cell isolation

Isolated cardiac myocytes were prepared by use of an enzymatic perfusion method (Sánchez-Chapula, 1988). Adult cats (1.5–3 kg) were anticoagulated with heparin (1000 u kg^{-1}) and anaesthetized with pentobarbitone sodium (35 mg kg^{-1}, i.p.). The hearts were excised and perfused via the aorta in a Langendorff system for retrograde coronary perfusion. Hearts were initially perfused with Tyrode solution for 5 min, followed by perfusion with nominally zero-calcium Tyrode solution for another 5 min. The perfusate was changed to zero-calcium Tyrode solution for another 5 min. The perfusate was changed to zero-calcium solution containing collagenase (1 mg ml^{-1}; type I, Sigma Chemical) and protease (0.1 mg ml^{-1}; type XIV, Sigma Chemical) for 35 min. The enzymes were washed out by perfusion with a solution high-potassium, low-sodium, low-chloride ('KB-medium'; Isenberg & Klockner, 1982) for a period of 5 min. Thin layers of epicardial or endocardial tissue were dissected out from left ventricular free wall. Single cells were obtained by mechanical agitation with a pipette. The cells were stored in the 'KB-medium' at 4°C for later electrophysiological experiments. The Tyrode solution had the following composition (mM): NaCl 125, $NaHCO_3$ 24, NaH_2PO_4 0.42, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, glucose 11.0 and taurine

10; the solution was equilibrated with 95% O_2: 5% CO_2 (pH 7.4). Nominally zero-calcium solution was prepared simply by omitting $CaCl_2$ from the Tyrode solution. The high-potassium, low-sodium, low-chloride solution ('KB-medium') had the following composition (mM): K-glutamate 60, KCl 50, taurine 20, KH_2PO_4 3, glucose 10, HEPES 10, ethyleneglycol-*bis* (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) 0.2; pH was adjusted to 7.4 with KOH.

Whole cell current- and voltage-clamp technique in single cells

All experiments were performed at 35°C. Isolated single cells were placed in a small-volume (0.2 ml) recording chamber on the stage of an inverted microscope (Diaphot, Nikon). Action potentials under current-clamp conditions and macroscopic current recordings under voltage clamp conditions were obtained with the whole cell method by use of an amplifier (Axopatch 1C, Axon Instruments). Glass pipettes had tip resistances of 2–4 MΩ when filled with internal solution during action potential experiments. In voltage clamp experiments the tip resistances were 1.5–2 MΩ. The resistance in series with the cell membrane was compensated to provide the fastest possible capacity transient without current oscillations. Liquid junction potential (−9 mV) between the pipette and the bath solution was always corrected. Currents were filtered with a four-pole Bessel filter at 2 kHz, digitized at 4 kHz, and stored on an Epson 486 DX/33 computer (pClamp Software, Axon Instruments).

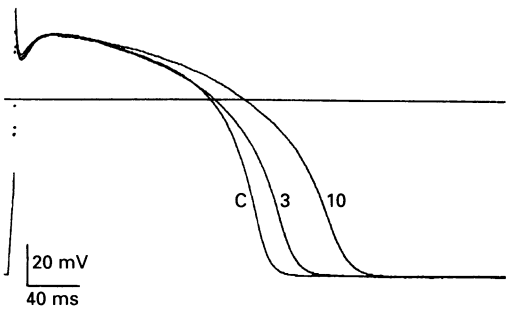

Figure 1 Effect of berberine on action potential of cat ventricular myocyte (BCL = 1000 ms; temperature 35°C). Shorter action potential was obtained under control conditions (C). After 8 min of application of berberine 3 μM (3) and 10 μM (10) to the bath, action potential duration was prolonged without affecting resting potential or amplitude of the action potential.

The normal external solution had the following composition (mM): NaCl 140, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 1.0, HEPES 10 and glucose 11; pH was adjusted to 7.4 with NaOH. The Ca^{2+}-Co^{2+} external solution had the following composition (mM): NaCl 140, KCl 4, $CaCl_2$ 0.5, $CoCl_2$ 2.0, $MgCl_2$ 1.0, HEPES 10 and glucose 11; pH 7.4 with NaOH. The internal solution had the following composition (mM): K-aspartate 80, KCl 40, KH_2PO_4 10, $MgSO_4$ 1, Na_2ATP 5, HEPES 5 and EGTA 5, pH 7.3 with KOH. In experiments designed to study the effect of berberine on calcium current, the external solution had the following composition (mM): NaCl 140, CsCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 10, glucose 11; pH 7.4 with NaOH.

The internal solution had the following composition (mM): Cs-aspartate 80, CsCl 50, $MgSO_4$ 1, Na_2ATP 5, HEPES 5, EGTA 5, nystatin 150 μg ml^{-1}; pH 7.3 with CsOH. Berberine chloride (Sigma Chem.) was dissolved directly in the external solutions. Nystatin was dissolved in dimethylsulphoxide at a concentration of 25 mg ml^{-1}. Data are expressed as means ± s.d. Statistical significance was evaluated by Student's *t* test, where appropriate. Differences were considered significant at $P<0.05$.

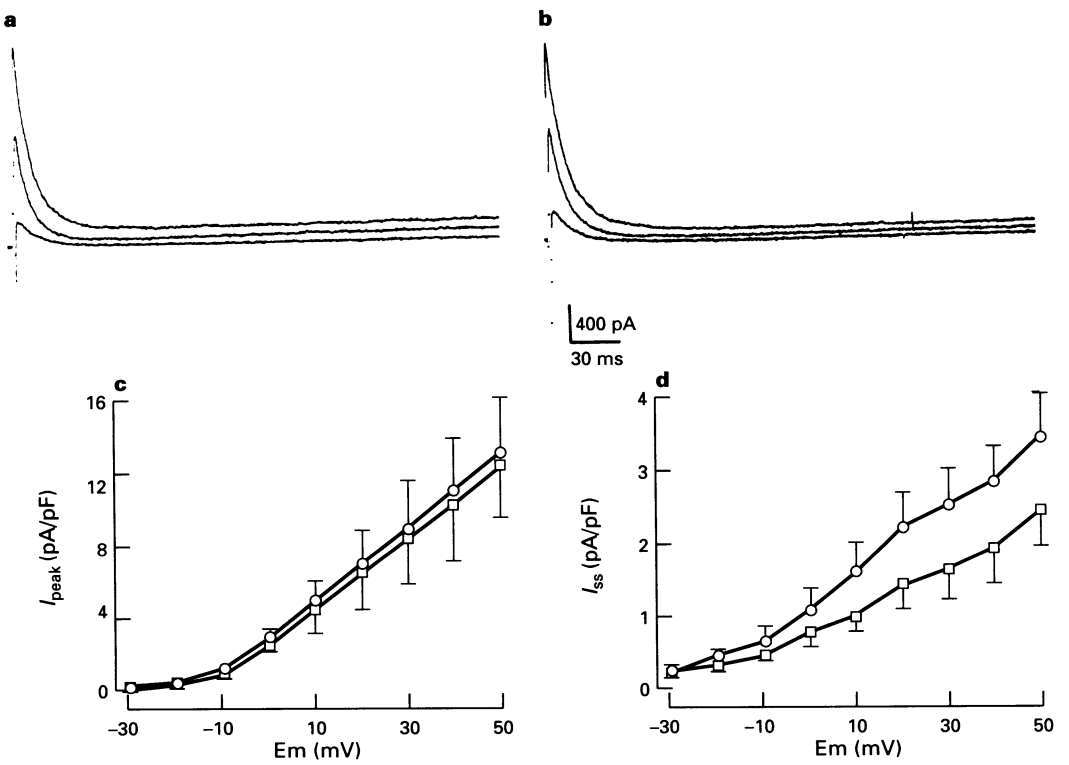

Figure 2 Effects of berberine 10 μM on membrane currents of cat epicardial ventricular myocytes at 35°C. (a) Depolarizing steps of 300 ms applied at 0.1 Hz, from −70 mV to −10, +10 and +30 mV, produced rapidly inactivating outward currents followed by a small, slowly activating outward current. (b) Berberine produced a decrease (8%) in the peak current and induced a stronger decrease on the current measured at the end of the pulse. (c) Current-voltage relation for the peak outward current under control conditions and in the presence of berberine. (d) Current-voltage relation for the current measured at the end of the depolarizing pulse ($n=6$ cells): (○) control; (□) berberine 10 μM.

Results

In the first series of experiments, performed at 35°C, the effect of berberine (3 and 10 μM) was studied on action potential characteristics in cat single ventricular myocytes applying pulses of 5 ms of duration, at a basic cycle length (BCL) of 1000 ms, using the nystatin method (Figure 1). The resting membrane potential, action potential amplitude and plateau phase were not significantly modified by the drug. The most striking effect of the drug was an increase in action potential duration. The effect was complete after 8 min of superfusion with the drug. In 5 cells, berberine significantly ($P<0.05$) increased action potential duration measured at 90% of repolarization (APD_{90}) from 188 ± 29 ms to 224 ± 23 ms at 3 μM and 278 ± 33 ms at 10 μM (Figure 1). The effect of the drug was poorly reversible after 15 min of washout. APD_{90} 243 ± 31 ms (data not shown).

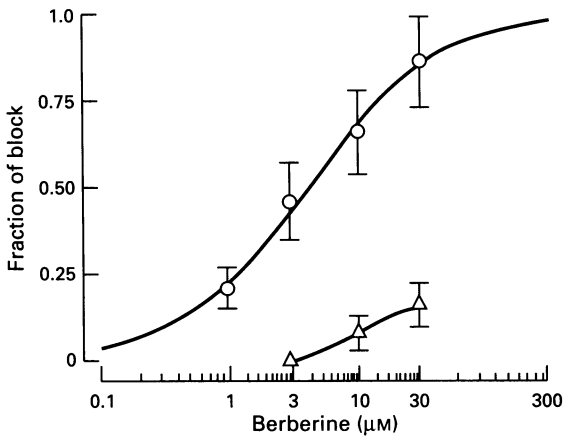

Figure 3 Concentration-response curves for the blocking effects of berberine on I_K (○) and I_{to1} (△). I_K block was measured as the decrease in tail current amplitude at −40 mV after depolarizing pulses of 3 s of duration to +30 mV. I_{to1} block was measured as the decrease in peak transient outward current amplitude during depolarizing pulses from −80 to +50 mV. Data of I_K block were fitted by the equation:

$$f = 1/1 + (K_d/[D])^{n_H}$$

where K_d is the dissocaition constant and n_H is the Hill coefficient. K_d was 4.1 μM and $n_H = 0.94$.

Figure 2 shows the effect of 10 μM berberine on membrane currents in an epicardial cell. The experiment was performed at 35°C. These cells show a prominent calcium-independent transient outward potassium current (I_{to1}) followed by a small delayed rectifying outward current (I_K). The drug at 10 μM decreased the peak outward current measured at +50 mV by $8\pm5\%$, ($n=6$ cells). Berberine significantly decreased the current measured at the end of the depolarizing pulse to +50 mV by $31\pm11\%$ at 10 μM ($n=6$), suggesting a stronger inhibition of the delayed rectifying outward current. The decrease in the peak outward current may be due to reduction of the steady-state current. Berberine did not modify the apparent inactivation time course of the transient outward current.

In Figure 3 the concentration-response relationship for the effects of berberine on I_K was measured as a decrease in tail current amplitude at −40 mV, after a depolarizing pulse of 3 s to +30 mV. The effect on I_{to1} was measured as the decrease in peak amplitude of the transient outward current induced by a depolarizing pulse to +50 mV. Berberine was significantly more potent in blocking I_K than peak outward current. The K_d for the effect on I_K was 4.1 μM. The maximum effect on peak transient outward current at 30 μM was $16\pm6\%$.

The effects of berberine at concentrations between 0.3 and 30 μM was studied on the inward rectifying current (I_{K1}). In Figure 4 we show currents obtained by applying test pulses from −40 mV to potentials between −100 and −30 mV. Current traces obtained under control conditions (Figure 4a) and in the presence of berberine 30 μM (Figure 4b). The drug did not modify the current amplitude. Current-voltage re-

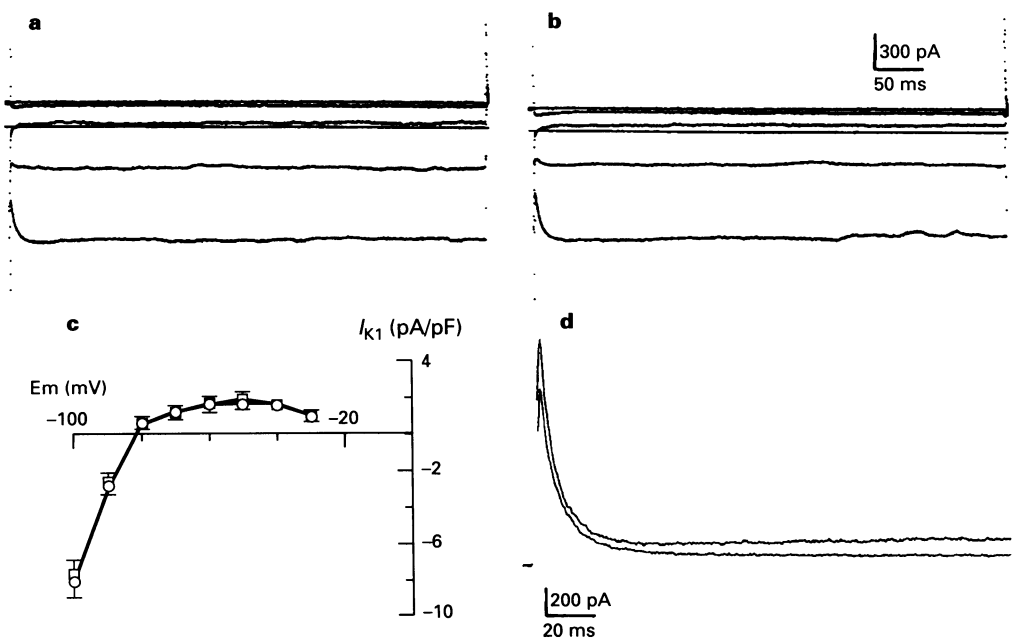

Figure 4 Berberine did not modify I_{K1}. (a) Current traces obtained under control conditions by applying clamp pulses of 500 ms of duration to potentials of −100, −80, −70, −50 and −30 mV from an HP of −40 mV. (b) Current traces obtained in the presence of berberine 30 μM. (c) Current-voltage relationship of the current measured at the end of the test pulses under control (○) and in the presence of berberine 30 μM (□). (d) Superimposed current traces induced by applying a depolarizing pulse to +30 mV from an HP of −80 mV at a frequency of 0.1 Hz, under control conditions and in the presence of berberine 30 μM (same cell as in panels a and b).

lationships of the current measured at the end of the 500 ms pulses under control and in the presence of berberine 30 μM are shown in Figure 4c. Berberine did not modify I_{K1} at any of the concentrations used between 0.3 and 30 μM. In the same cell as in Figure 4a and b, the effect of berberine 30 μM on current induced by a 200 ms duration depolarizing pulse at a frequency of 0.1 Hz, to +30 mV from a HP of −70 mV is shown (Figure 4d). Berberine at this concentration significantly decreased the peak outward current and the current measured at the end of the pulse.

The effect of berberine (0.3−30 μM) on the high threshold calcium current ($I_{Ca\text{-}L}$) was studied in cat ventricular myocytes. Experiments were performed at 35°C. In order to minimize potassium currents, caesium instead of potassium was used in the internal and external solutions. Rundown of $I_{Ca\text{-}L}$ was prevented by use of the nystatin method (Kurachi *et al.*, 1989). Berberine did not modify $I_{Ca\text{-}L}$ at any of the concentrations used. In Figure 5 are shown current traces induced by depolarizing pulses from −40 mV to −20, −10, 0 and +10 mV,

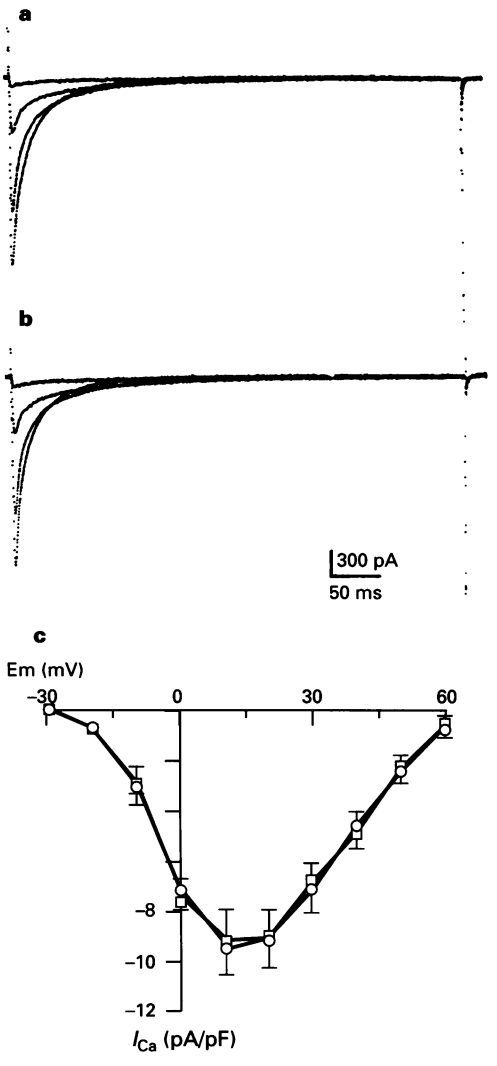

Figure 5 Effect of berberine on $I_{Ca\text{-}L}$. The effect of berberine 30 μM was studied on the high threshold calcium current using the nystatin method, caesium was used instead of potassium in the external and internal solution; temperature 35°C. From a HP of −40 mV depolarizing pulses of 500 ms of duration were applied to potentials between −30 and +50 mV at a frequency of 0.1 Hz. (a) Current traces induced by depolarizing pulses to −30, −20, −10 and 0 mV under control conditions. (b) Current traces obtained in the presence of berberine 30 μM. (c) Current-voltage relationship for the peak inward current under control conditions (○) and in the presence of berberine 30 μM (□) ($n=5$ cells).

under control conditions (Figure 5a) and in the presence of berberine 30 μM (Figure 5b). Summarized results obtained from five cells are shown in the *I-V* relationship of $I_{Ca\text{-}L}$ of Figure 5c. Berberine at 30 μM did not modify $I_{Ca\text{-}L}$ at any of the potentials studied.

To study the effect of berberine on I_K in more detail, endocardial cells were used which display no (or minimal) I_{to} (Furukawa *et al.*, 1990). Figure 6 shows the effect of berberine 3 μM on membrane currents from an endocardial cell. The currents were induced by application of 3 s depolarizing pulses to potentials between −30 and +50 mV, from a holding potential of −40 mV. At the beginning of the depolarizing pulse the current is less outward with respect to the holding current at −40 mV, but slowly develops in the outward direction. On repolarization each current trace is followed by a slowly decaying current tail. This current has been identified as the delayed rectifying current (I_K) (Follmer & Colatsky, 1990; Furukawa *et al.*, 1992). Berberine decreased the current activated during the depolarizing pulse and the subsequent tail current. Figure 6c shows the effect of berberine (3 μM) on tail currents after step depolarizations between −30 and +50 mV. Analysis of the activation curves (Figure 6c) indicates that the block increased with stronger depolarizations, suggesting that the block was voltage-dependent. The voltage-dependence of channel opening is described by the Boltzmann equation:

$$I_K(\text{tail}) = I_K(\text{tail})\text{max}/\{1+\exp[V_{0.5}-V)/K]\}$$

where I_K(tail) is the tail current measured at −40 mV after 3 s activating pulses to voltages between −30 and +50 mV, $V_{0.5}$ is the membrane potential at which 50% of the channel openings occur, I_K (tail)max is the fitted maximal current, V is the activating membrane voltage, and K is the slope factor. The following values ($n=5$ cells) were found: under control conditions half the maximum voltage was 11.1±1.7 mV and the slope factor was 6.9±0.9 mV. In the presence of berberine 3 μM, $V_{0.5}$ was 3.3±1.8 mV and K was 6.4±1.1 mV. The voltage-dependence of the block is more clearly shown in Figure 6d. The degree of block significantly increased with larger depolarizations. The maximal effect was attained at +30 mV, which is the level of maximal activation of I_K (Figure 6c). The blocking effect was similar for voltages between +30 and +50 mV, suggesting that there is no intrinsic voltage-dependence of the block (there is no further block at positive potentials once all channels are activated).

From the above results it is clear that berberine mainly blocks I_K. In order to gain more insight into the mechanism of this block, the rest of the work focuses on the effect of the drug on I_K. The voltage-dependent increase of block between −30 and +30 mV suggests that berberine could be an open stage channel blocker (Snyders *et al.*, 1992). In order to obtain additional evidence about possible open channel block by berberine, the time-dependent development of block with the drug was studied using protocols previously described (Carmeliet, 1993). The change in amplitude of tail currents after clamp pulses of different durations was measured under control conditions and in the presence of berberine. Examples of such records for depolarizing steps to +30 mV of 100, 300, 1100 and 1900 ms of duration applied every 2 min are shown in Figure 7 under control, and in Figure 7b in the presence of berberine 3 μM. Under control conditions, pulses of increasing duration yielded time-dependent and tail currents of increasing amplitude, reaching a plateau in 2 s. In the presence of berberine 3 μM, after a 100 ms pulse, the tail current decreased by 26% of that under control conditions. The block was increased to 31% after a 300 ms pulse, to 62% after a 1100 ms pulse, and remained at 62% after a 1900 ms pulse. The relative amount of block was estimated by calculating the ratio of tail amplitude in the presence and in the absence of three different concentrations of the drug (0.3, 3 and 10 μM). The time course of block development was quantified by plotting these ratios as a function of clamp duration (Figure 7c). These results show that

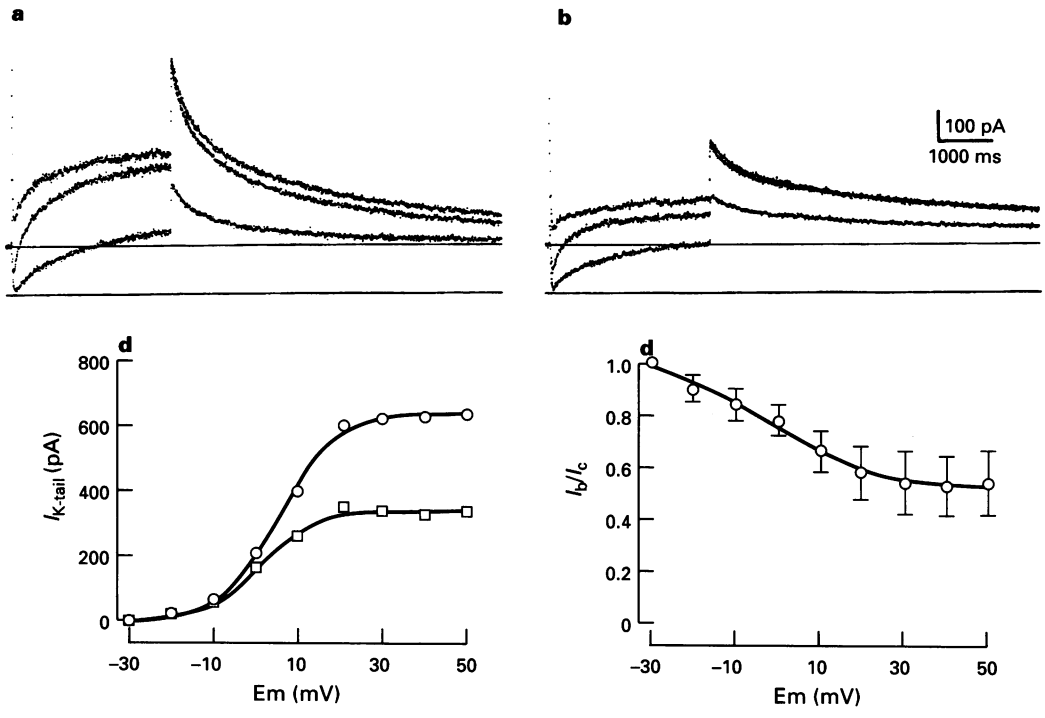

Figure 6 Effects of berberine on I_K of cat endocardial ventricular myocytes. (a) Membrane currents induced by 3 s depolarizing pulses to 0, +20 and +40 mV from HP of −40 mV, under control conditions. (b) Membrane currents obtained in the presence of berberine (3 μM). The lower solid line is zero current level. The upper solid line is holding current at −40 mV. (c) Voltage-dependent tail current activation under control conditions (○) and in the presence of berberine (3 μM) (□). All activation data were fitted by the Boltzmann equation. (d) The relative tail current amplitude after drug is plotted for each test potential as a fraction of the tail amplitude measured under control conditions. The line through the experimental data is the best fit with the Boltzmann equation.

block of I_K by the drug consisted of two components. There was an instantaneous component followed by a time-dependent component. Both the relative magnitude of the instantaneous component and the rate of block development and magnitude of the time-dependent component of inhibition increased when the berberine concentration was increased.

If berberine interacts with the open channel, then dissociation of berberine from the blocked channel should result in a transient conducting channel which subsequently could close. Figure 8 shows the superposition of the currents induced by depolarizations of 3 s duration to 0 mV and the tail currents at −40 mV, under control conditions and in the presence of berberine 3 μM. The holding potential was −40 mV. Pulses were applied at a frequency of 0.1 Hz. In the absence of the drug, this depolarization activated I_K which then decayed upon repolarization to −40 mV. The drug induced a decrease in current during the depolarising pulse and peak amplitude of the tail current. Current measured at the end of the depolarizing pulse was 372 pA for the control and 272 pA in the presence of berberine; the peak tail current amplitude was 408 pA in the control and 265 pA in the presence of berberine. In addition, it slowed the decay of the tail current. This resulted in a 'crossover' phenomenon. Under control conditions, after depolarizing pulses to +30 mV tail currents declined to half of the peak amplitude ($t_{½}$) in 482±55 ms ($n=7$). After exposure to berberine (3 μM), the tail current declined with $t_{½}=883\pm$ 327 ms. The slowing in tail current decay and the crossover phenomenon are compatible with transient unblocking and provide additional evidence for open channel block.

The possibility of use-dependence was explored in the next set of experiments. In Figure 9, the upper current trace was obtained before the administration of the drug under steady-state conditions at a frequency of 0.5 Hz. After 10 min of superfusion with berberine 10 μM (without stimulation) a train of 64 pulses at a frequency of 0.5 Hz was applied. The middle and lower records of Figure 9 show the 1st and 64th current traces of the train, respectively. It is clear that berberine decreased the current activated during the first pulse to +30 mV and the tail current. Tail current after the first pulse in the presence of berberine decreased to 67±9% of the control. In addition, a use-dependent effect was observed during the train and the tail current inhibition increased to 39±7% of control, with a τ of 0.61 pulses.

In order to study possible rate-dependent effects of berberine, the steady-state currents under control conditions (Figure 10 upper traces) and in the presence of berberine (lower traces) were activated during 200 ms pulses to +30 mV and tail currents measured at −40 mV. Pulses were applied every 2.5 s (Figure 10a) and every 0.8 s (Figure 10b). The inhibition induced by the drug was similar at both frequencies.

In guinea-pig atrial and ventricular myocytes I_K is composed of two different delayed rectifiers I_{Kr} and I_{Ks} (Sanguinetti & Jurkiewicz, 1990; 1991). Recently, similar results have been found in dog and rabbit ventricular myocytes (Liu & Antzelevitch, 1995; Salata *et al.*, 1995). Dofetilide is a class III antiarrhythmic drug that selectively blocks I_{Kr} (Jurkiewicz & Sanguinetti, 1993). In Figure 11 the effect of a high concentration of dofetilide (5 μM) and dofetilide plus berberine on I_K are shown. Figure 11a shows control current traces to potentials between −30 and +50 mV from a HP of −40 mV. In Figure 11b the current traces obtained in the presence of dofetilide 5 μM are shown and in Figure 11c the current traces in the presence of dofetilide 5 μM plus berberine 30 μM. Current-voltage relationships for the effects on time-dependent current activated during the depolarizing pulse (Figure 11d) and tail current amplitude (Figure 11e) are shown under control conditions, with both dofetilide alone and dofetilide plus berberine. Dofetilide 5 μM produced a significant block of the current activated during the depolarizing pulse and tail current. However, in the presence of this concentration of dofetilide which has been shown to be sufficient to block I_{Kr} in guinea-pig ventricular myocytes (Jurkiewicz & Sanguinetti, 1993), a significant current remained. At the higher concentration used in

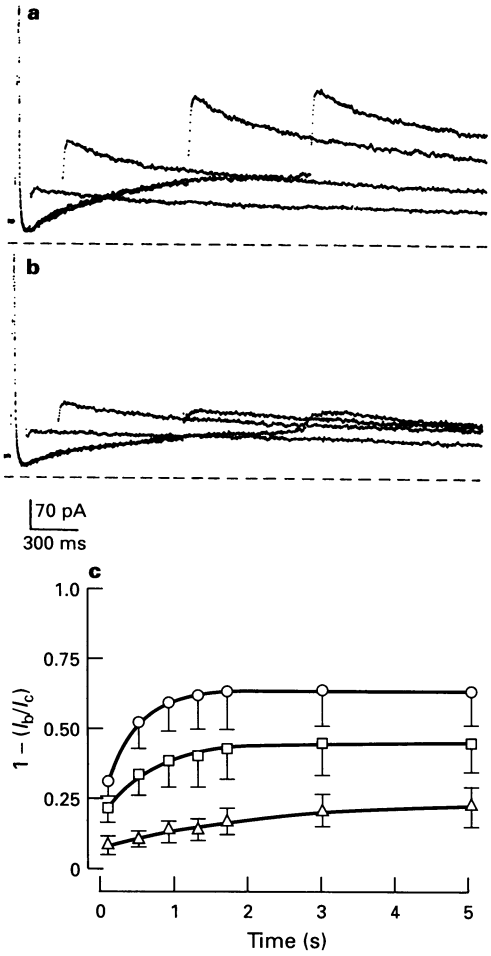

Figure 7 Development of block estimated by the tail current amplitude. Depolarizing pulses from −40 to +30 mV of various durations were applied every 2 min. Superimposed currents during and after (tails) depolarizing pulses are shown under control conditions (a) and in the presence of berberine 3 μM (b). Note that this cell presented a prominent transient outward current at the beginning of the depolarizing pulse that was not affected by the drug. In (a) and (b) the interrupted line is zero current level. (c) Time course of block development with three different concentrations of berberine. Ratios of tail current amplitude in the presence of the drug and under control conditions are plotted against the duration of the pulse. The curves are fitted by a single exponential plus a constant offset. The offset value was 0.06±0.02 at berberine concentration of 0.3 μM (△), 0.19±0.04 at 3 μM (□) and 0.26±0.04 at 10 μM (○). The time constants were 3.5 s at berberine 0.3 μM, 0.7 s at 3 μM and 0.39 s at 10 μM.

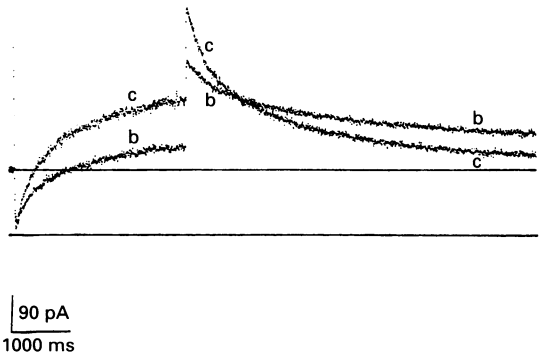

Figure 8 Berberine slowed decay of tail current and induced current crossover. Berberine (3 μM) reduced time-dependent current induced by a depolarizing pulse to +10 mV. In addition, reduced peak tail current amplitude measured at −40 mV and the decline of the tail current was slower, resulting in 'crossover' of the currents. Holding potential was −40 mV, pulses were applied at a frequency of 0.1 Hz; temperature 35°C. The lower solid line is zero current level; the upper solid line is holding current at −40 mV.

the present work, berberine (30 μM) did not significantly modify either dofetilide-resistant current activated during the depolarizing pulse or tail current. Similar results were found in four different cells. If we assume that the dofetilide-resistant current is I_{Ks} (Jurkiewicz & Sanguinetti, 1993), berberine did not modify I_{Ks}.

Discussion

The results of this study show that in cat isolated ventricular myocytes, berberine prolongs action potential duration without affecting resting membrane potential or action potential amplitude. Similar results have been reported for multicellular

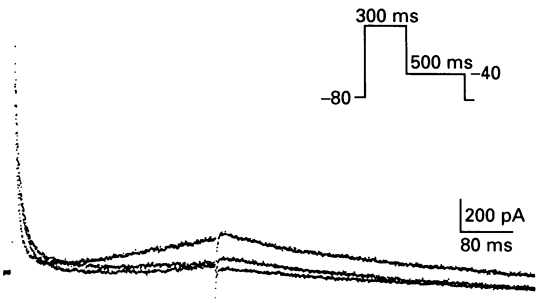

Figure 9 Tonic and use-dependent effects of berberine. Superimposed current traces induced by depolarizations of 300 ms of duration, from −80 to +30 mV and tail current at −40 mV. Control current (upper trace) was obtained under steady state conditions at a frequency of 0.5 Hz. Application of pulses was stopped and berberine 10 μM superfused for 10 min. A train of 64 pulses was then applied at a frequency of 0.5 Hz. The middle and lower traces were obtained during the first and 64th pulses of the train, respectively.

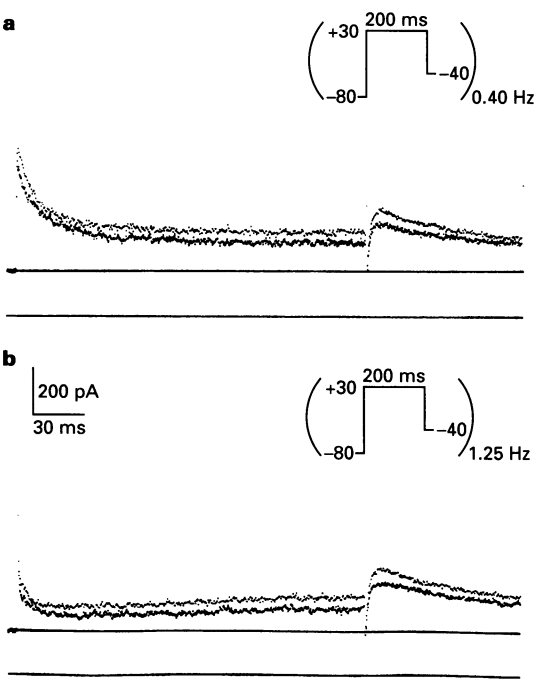

Figure 10 Frequency-dependent effects of berberine. Superimposed current traces under steady state conditions, in the absence and presence of berberine 10 μM. Depolarizing pulses were applied from −80 to +30 mV for 200 ms, then voltage was set at −40 mV for 100 ms, at a frequency of 0.4 Hz (a) and 1.25 Hz (b). The blocking effect of berberine on the current activated during the depolarizing pulse and tail current measured at −40 mV was similar at both frequencies. In both panels the lower solid line is zero current level and the upper solid line is holding current at −40 mV.

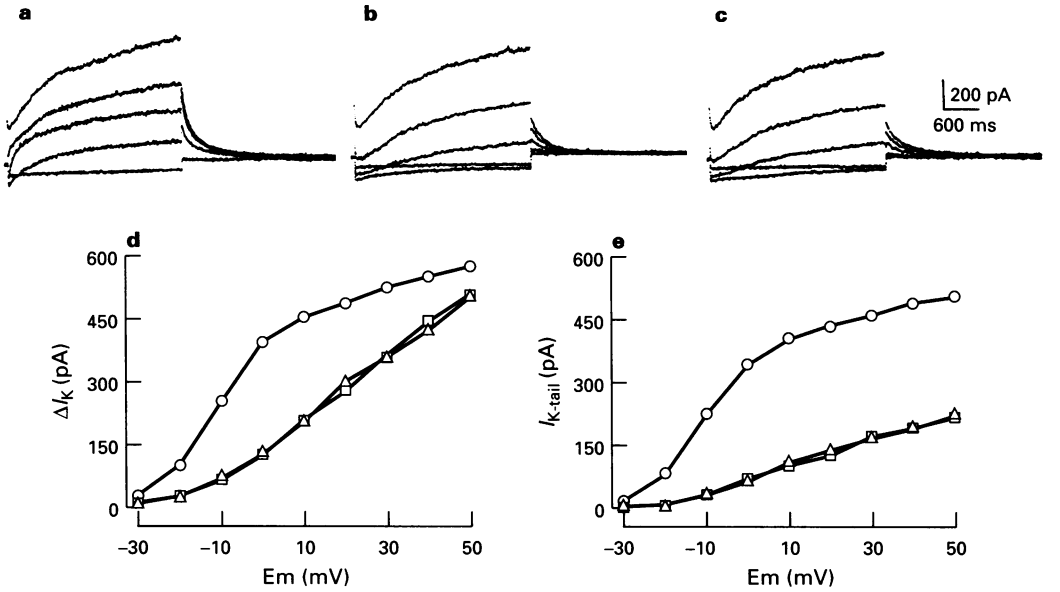

Figure 11 Effects of dofetilide and dofetilide plus berberine on I_K. From an HP of −40 mV depolarizing pulses of 3 s of duration to potentials between −30 and +50 mV were applied at 0.1 Hz (temperature 35°C). Current traces induced by pulses to −30, −10, +10, +30 and +50 under control conditions (a), in the presence of dofetilide 5 μM (b), and in the presence of dofetilide 5 μM plus berberine 30 μM (c). (d) Current-voltage relationship of the time-dependent current activated during the depolarizing pulse under control (○), dofetilide (□) and dofetilide plus berberine (△). (e) Current-voltage relationship of the peak tail current under each of the three experimental conditions.

preparations (Huang *et al.*, 1990; Ricciopo Neto, 1993). In dog Purkinje and ventricular muscle fibres, berberine at concentrations of 3–30 μM did not affect maximum upstroke velocity (V_{max}; Ricciopo Neto, 1993). These findings and ours show that at these concentrations berberine selectively increases action potential duration.

Our results show that berberine, at concentrations of 0.3 to 3 μM, inhibits only I_K. At higher concentrations (10–30 μM) this drug also decreased the peak outward current. This effect could be explained by a decrease in I_{to}. However, it may also be partially due to the decrease in I_K. At the concentrations used (0.3–30 μM) the drug did not affect I_{K1} or I_{Ca}.

In guinea-pig ventricular and atrial myocytes the delayed rectifier outward current (I_K) has been described as the sum of two overlapping outward currents, I_{Ks} and I_{Kr} (Sanguinetti & Jurkiewicz, 1990; 1991). In cat ventricular myocytes Furukawa *et al.* (1992) using an 'envelope of tails test' found that I_K was composed of only one component of current. In addition, I_K in cat ventricular myocytes was blocked by the specific I_{Kr} channel blocker, E-4031 (Follmer & Colatsky, 1990). In the present work, we have found a dofetilide-resistant component of current, which could be interpreted as I_{Ks}. This dofetilide-resistant delayed rectifying current was not affected by berberine, suggesting that berberine selectively blocked I_{Kr}. However, further evidence is required to probe the presence of two different delayed rectifier currents in cat ventricular myocytes. I_K in cat ventricular myocytes has been shown to be blocked by antiarrhythmic agents such as flecainide (Follmer & Colatsky, 1990) and WAY-123,398 (Spinelli *et al.*, 1993). However, in those studies I_K has been considered to be composed of a single kind of delayed rectifier.

The effect of berberine on I_K shows a tonic component, that is, the drug already reduces the current during the first of a train of pulses applied after a rest period of 10 min. In addition, a use-dependent effect is present, since the blocking effect of the drug is increased during the train of pulses. The I_K inhibition induced by berberine in the range of voltages between −30 and +30 mV suggests a voltage-dependent modulation of the block. However, this block in itself is voltage-independent, since no further block is observed once all the channels are activated.

Shifts of the activation or inactivation curves can occur when a drug preferentially blocks the channel in a given state (Hille, 1978). In the present work, evidence has been found that berberine blocks open channels. Possible explanations for the tonic block may be that the drug also blocks the channel in the rested state or that there is fast component of block to open or intermediate closed channels, in addition to the time-dependent open channel block. The decay of the tail current after an activating depolarizing pulse mainly reflects the irreversible closing of the channel. If a drug blocks the open channel, the dissociation of the drug results in a conducting channel. Blocked channels are not conducting and the conversion to open conducting channels should, therefore, result in a rising phase of the tail current. Thereafter the tail current should display a slower decline because some fraction of the open channels become blocked again, rather than closing irreversibly (Armstrong, 1971; 1990; Snyders *et al.*, 1993). These two predictions were fulfilled in the present experiments with berberine (Figure 6), providing evidence that the berberine-induced inhibition of I_K is at least partially due to an open channel block. Further evidence for open channel block is provided by the experiments using pulses of different durations and measuring the amplitude of the tail currents (Figure 7). These experiments again show a tonic component that is increased with berberine concentration. In addition, the block is increased with the duration of the pulse. The time-dependent development of block is faster, the higher the berberine concentration. Nevertheless, use-dependent effects are obvious with berberine; no frequency-dependent effects are observed at the frequencies explored in the present work (0.4–1.25 Hz). This can be explained on the basis of a very slow recovery from block at the resting potentials and/or a relatively short time constant for block development (Carmeliet, 1993). Similar results have been found with other drugs such as almokalant and dofetilide (Carmeliet, 1992).

In summary, the results of the present work show that the increase in action potential duration induced by berberine is mainly due to its blocking effects on I_K. The effects of the drug on I_K are a tonic inhibition, explained by rested state channel block or a fast block or intermediate closed channels and a slower time-dependent inhibition due to open channel block.

This open channel block induces use-dependent block. However, it does not induce frequency-dependent block. Prolongation of action potentials by berberine on multicellular preparations has been shown to be greater at low stimulation frequencies (Ricciopo Neto, 1993). However, this effect cannot be explained by reverse use-dependent block on I_K.

The author wishes to thank Dr Dirk Snyders for critical reading of the manuscript, Gusti Gould de Pineda for editorial assistance, Juan Fernando Fernandez for technical assistance and Juan Carlos Muñoz for preparing the Figures. This work was supported by CONACyT (Mexico) Grant 2004-M9301.

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(Received June 5, 1995
Revised November 13, 1995
Accepted December 7, 1995)