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Mixed blockade of K^+ and Na^+ currents in rat ventricular myocytes by the tedisamil analogue, KC8851

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Abstract

The whole-cell voltage clamp method has been used to study the actions of KC8851, a structural analogue of tedisamil (KC8857), on the transient outward K^+ current (I_{to}) and the Na^+ current (I_{Na}) in rat ventricular myocytes. KC8851 diminished the inactivation time course of I_{to} with an EC_{50} value of 2.2 μM. Kinetic analysis of the time-dependent effects of KC8851 on the decay of I_{to} was consistent with open channel blockade with respective blocking and unblocking rate constants of 9.3×10^6 M^{-1} s^{-1} and 19.3 s^{-1}. KC8851 was also found to be a potent inhibitor of peak Na^+ current with an EC_{50} value of 5.1 μM. The inhibition of I_{Na} was primarily use-dependent with some contribution from tonic block. The results show KC8851 exhibits potent and selective mixed blockade of I_{to} and I_{Na} a result different from that previously documented for the analogue, tedisamil, which was found to be a potent blocker of I_{to}, but not I_{Na}, in rat ventricular cells. © 1997 Elsevier Science B.V.

Keywords: Patch clamp, whole-cell; K^+ current; Na^+ current; KC8851; Open channel blockade

1. Introduction

The dihydrochloride compound KC8851 is structurally related to tedisamil (KC8857), a potent blocker of transient outward K^+ current (I_{to}) in rat ventricular myocytes (Dukes and Morad, 1989; Dukes et al., 1990) and delayed rectifier K^+ current in guinea pig ventricle (Dukes et al., 1990; Ohler et al., 1994). In rat, tedisamil was highly selective for block of K^+ current; significant effects to inhibit inward Na^+ current (I_{Na}) were not observed until concentrations of the agent were increased to levels in excess of 20 μM (Dukes and Morad, 1989).

The properties of KC8851 on ECG measures, electrical stimulation and antiarrhythmic effects were examined in vivo (McLarnon et al., 1996; Xu et al., 1997). The compound, at low doses, exhibited antiarrhythmic activities against both electrically and occlusion-induced arrhythmias. KC8851 modified variables such as QT intervals and effective refractory periods which depend on K^+ current. Of interest, however, was the finding that at low concentrations the compound also altered the Na^+-dependent parameters of PR interval and RSh (measured as the height from the peak of the R wave to the bottom of the S wave). Both quantities have been suggested as sensitive indices of the blockade of Na^+ channels (Penz et al., 1992). Thus KC8851, unlike tedisamil, may also be a potent inhibitor of inward Na^+ current. This point is important given the possibility that mixed block of K^+ and Na^+ currents, at low drug concentrations, may serve as an effective antiarrhythmic strategy (Colatsky, 1995).

The essential objective of the present experiments in isolated rat ventricular myocytes was to determine the potency and actions of KC8851 on properties of I_{to} and I_{Na}. In the former case, a number of patch clamp protocols have been applied to determine interactions of KC8851 with the closed, open and inactivating states which describe the operation of I_{to}. In particular, a kinetic analysis of the effects of KC8851 on the time course of I_{to} was used to quantitate the rate constants associated with open channel blockade. The potency for KC8851 inhibition of peak Na^+ current was also investigated to ascertain if the compound exhibited mixed block of both K^+ and Na^+ channels. In addition, effects of KC8851 on the voltage dependence of activation and inactivation of I_{Na} and possible use-dependent block were also studied. We also wished to establish whether KC8851 was selective for actions on I_{to} and I_{Na}. This point was addressed by determining that a single concentration of KC8851, at 20 μM, had no effect

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on either inward rectifier I_{K1} or inward I_{Ca} which contribute to the time course of the repolarization of the ventricular action potential. The results of this work have shown that KC8851, unlike its analogue tedisamil, exhibits potent mixed blockade of I_{to} and I_{Na} in rat ventricular myocytes.

2. Materials and methods

2.1. Isolation of rat ventricular myocytes

The rat ventricular myocytes were dissociated and isolated from male rats (Sprague-Dawley, 250–350 g) using procedures which have been described previously (Mitra and Morad, 1985; McLarnon and Xu, 1995). Briefly, rat heart was removed from pentobarbitone anesthetized animals and was perfused with a constant-flow Langendorff system with a Tyrode's solution containing (mM): NaCl (133.5), KCl (4), $MgCl_2$ (1.2), NaH_2PO_4 (1.2), TES (10) and glucose (11), pH at 7.4. The perfusate was oxygenated for 30 min and maintained at 37°C. After 4 min, the perfusate was exchanged for another solution containing 0.07% collagenase (Type II, Worthington Biochemical), $CaCl_2$ (25 μM) and 200 mg of bovine serum albumin (BSA, Boehringer Mannheim). Following sufficient digestion, the heart was cut into small pieces which were then immersed in Tyrode's solution. After 15 to 20 min in this solution, gentle agitation was applied to further digest tissue into single cells. The cells were stored at room temperature, between intervals of wash with successively increased concentrations of Ca^{2+} (from 25 to 100 μM). Cells were plated on coverslips precoated with laminin (4 μg per coverslip) and used for electrophysiological study. In terms of morphology the cells had a mean length of 72 ± 6 μm ($n = 22$) and mean diameter of 7.8 ± 0.5 μm ($n = 22$).

2.2. Electrophysiology

Some of the procedures used in the recording of whole-cell currents from rat ventricular myocytes have been described previously (McLarnon and Xu, 1995). In the present work, macroscopic currents, primarily the transient outward K^+ current, I_{to}, and inward Na^+ current, I_{Na}, were recorded from isolated rat ventricular myocytes for periods up to 8 h after plating. Micropipettes, with resistances near 2 MΩ, were prepared using Corning 7052 capillary glass (A-M Systems, Everett, WA) and filled with a pipette solution with composition dependent on the particular experiment (see below). An Axopatch amplifier (200A, Axon Instruments, Foster City, CA) was used to record whole-cell currents with the low-pass filter set at 1 or 2 kHz. The sampling frequency of the A/D converter was 10 kHz. Capacitive currents and series resistance were compensated by use of analog circuitry and leak subtraction was also used in some experiments. The mean value of seal resistance was 36 ± 5 GΩ ($n = 12$) with series resistance compensation set at 80–85%, the mean series resistance was 4.2 ± 1 MΩ ($n = 10$) and the mean cell capacitance was 92 ± 6 pF ($n = 6$). P/N subtraction was not routinely done and there was no evident effect on macroscopic currents when it was employed. The protocols used were generated by computer (pClamp, Axon Instruments) and data were recorded on disk for subsequent analysis; unless noted the holding potentials were -70 mV. A detailed description of the specific protocols which were used in the work is included in the results section. In several experiments the effects of KC8851 (at 20 μM), on either I_{K1} or I_{Ca}, were studied with the particular protocols as described below. All experiments were performed at room temperature (20–24°C).

2.3. Data analysis

The data analysis used available software (pClamp). The inactivation time courses of I_{to} were best fitted with single exponential functions with associated time constants (denoted by τ). To determine the potency of KC8851 on I_{to}, dose–response curves were plotted for the effects of the compound to alter τ. In these plots the concentrations of KC8851 were represented linearly and the data were fitted with a single exponential function to obtain a value for the EC_{50}. The potency of inhibition of I_{Na} was determined from a dose–response plot where the response was measured as the effect of KC8851 to reduce peak current. The results in this study are given as mean value $\pm$ sem and statistical significance was determined using Student's *t*-test or two-way ANOVA. Statistical analysis was performed by the use of the NCSS statistical package. A difference at $P < 0.05$ was considered significant.

2.4. Experimental drug and solutions

The agent used in this study, KC8851, was obtained from Kali-Chemie (Hannover). The compound is a heterocyclic dihydrochloride with chemical structure shown in Fig. 1; it is a structural analogue of another Kali-Chemie compound, tedisamil (KC8857), with structure previously shown (Dukes et al., 1990; Ohler and Ravens, 1994). A fresh stock solution of the drug was prepared for each experiment (dissolved in distilled water) and KC8851 was

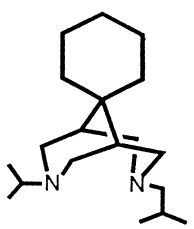

Fig. 1. Chemical structure of KC8851.

applied to the bath solution using a fast perfusion system for delivery.

In the experiments on I_{to} and I_{K1}, the bath solution was a modified Tyrode's solution and contained (mM): NaCl (133.5), KCl (4), $CaCl_2$ (1), $MgCl_2$ (1.2), TES (*N*-tris(hydroxymethyl)-methyl-2-aminoethanesulfonic acid, 10), NaH_2PO_4 (1.2) and glucose (11); pH adjusted to 7.35 with NaOH. The patch pipette solution contained (mM): NaCl (10), KCl (140), $MgCl_2$ (1), EGTA (10), Mg-ATP (5) and HEPES (10); pH adjusted to 7.35 with KOH.

For studies of I_{Na}, the bath solution contained 4 mM CsCl (to replace the KCl) and 50 mM NaCl with 87 mM Tris (to replace 133.5 mM NaCl). The pipette solution contained (in mM): NaCl (10), CsCl (120), EGTA (12), TES (10), $MgCl_2$ (1), Na-ATP (5); pH adjusted to 7.4 with CsOH.

Experiments on I_{Ca} used the following in the bath solution (in mM): Tris (137), $CaCl_2$ (5.5), $MgCl_2$ (1), CsCl (20) and glucose (5.5); pH adjusted to 7.2 with CsOH. The pipette solution used (in mM): CsCl (125), Mg-ATP (5), EGTA (15), TEACl (20) and HEPES (10); pH adjusted to 7.2 with CsOH.

3. Results

3.1. Inactivation time course of I_{to}

The initial experiments were designed to study the actions of KC8851 on the inactivation time course (τ) of I_{to} over a range of depolarizations to a maximum of +60 mV applied from a holding potential of −70 mV. In these experiments activation of I_{to} was observed with depolarizing steps more positive than −30 mV. A typical family of currents, evoked by depolarizing steps from the holding level to potentials up to +60 mV, is shown in Fig. 2a. The currents inactivated to steady-state levels with time constants exhibiting little or no dependence on potential. We follow the common convention that the inactivating and steady state macroscopic currents are separate entities (Apkon and Nerbonne, 1991; Castle and Slawsky, 1992; McLarnon and Xu, 1995) and that only the transient inactivating component, sensitive to 4-aminopyridine (4-AP), is termed I_{to}. The effects of KC8851 on the steady state current were not investigated, at present the identity of this current is not known (Apkon and Nerbonne, 1991). In Fig. 2b, a family of I_{to} are shown in the presence of KC8851 (at 2 μM). The time course of decay (τ) and the peak amplitude were diminished in the presence of the drug. Partial recovery of peak amplitude and τ after wash-off of KC8851 was found (Fig. 2c); full recovery to control levels was only obtained following prolonged wash-off of the compound.

A dose–response plot for the KC8851 effects to diminish the decay time course (τ) of I_{to} has been constructed (Fig. 2d). In $n = 8$ cells the mean value of τ, measured at +60 mV, was 43.3 ± 3.4 ms. Each point on the graph represents a normalized value from the experiments with application of 5 concentrations of KC8851 to each of the 8

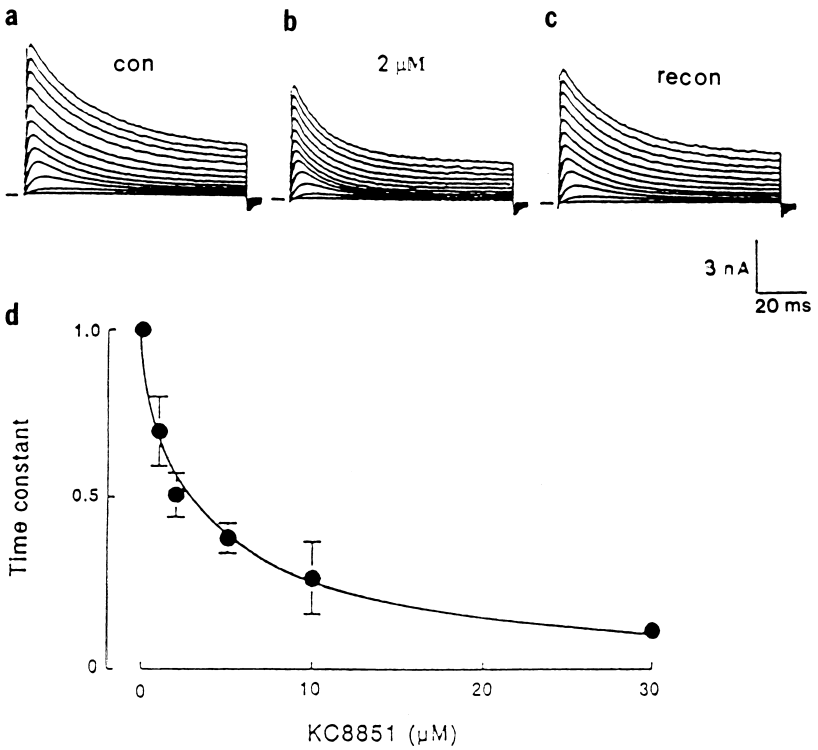

Fig. 2. Dose-dependent actions of KC8851 on I_{to}. (a) Family of I_{to} in control solution with depolarizing steps from −60 to +60 mV; holding potential was −70 mV. (b) Effects of 2 μM KC8851 on I_{to}. (c) Recovery of I_{to} after prolonged wash-off of KC8851. (d) Dose–response data for effects of 8851 on time constant τ. The data points are means ± sem from 8 cells and the solid line is a fit to the normalized data using a single exponential function.

cells. An individual value of τ was obtained by computer fitting, with a single exponential function, at the test potential of +60 mV. The resultant dose–response curve was fit by a decreasing exponential function and the concentration of KC8851 which reduced τ to 50% of control value (i.e., EC_{50}) was 2.2 ± 0.4 μM ($n = 8$). The actions of KC8851 to diminish τ, at 1 μM, were significant ($P < 0.05$). It should be noted that TTX was not used to block I_{Na}; the contribution of Na^+ influx to I_{to} would be very small with the protocols employed.

3.2. Voltage-dependence of activation of I_{to}

The effects of KC8851 on the voltage-dependence of activation of I_{to} were studied by measuring the peak currents at different test depolarizing potentials from the holding level of −70 mV. A plot of the peak current dependence on potential is presented in Fig. 3a for control (open circles) and with 2 μM KC8851 (closed circles). As evident from Fig. 3a, the amplitudes of I_{to} were decreased compared to control values once the current was activated. However, the thresholds for activation of I_{to} were close to −35 mV in the absence and the presence of the compound. Thus the voltage dependence of activation of I_{to} was not changed with KC8851; the same result was also obtained ($n = 6$ cells) at the highest concentrations of KC8851 30 μM used in this study (data not shown). The partial recovery of peak I_{to}, after wash-off of the drug, can also be noted in Fig. 3a (downward closed triangles).

3.3. Voltage-dependence of steady-state inactivation and recovery from inactivation of I_{to}

The interactions of KC8851 with the inactivated state of the I_{to} system was studied using a two-pulse protocol. In order to determine effects on the steady state inactivation of I_{to}, conditioning pulses from −90 to −30 mV (duration 300 ms) were followed with a single test pulse to +60 mV (duration of 200 ms). The normalized peak amplitudes of the I_{to} associated with the test pulse were then plotted vs. the conditioning voltages (Fig. 3b) where control data are represented by open circles and KC8851 effects (concentration of 2 μM) by closed circles. The resulting curves were fit by the Boltzmann equation:

$$\text{Normalized } I_{to} = 1/\left\{1 + \exp\left[\left(V - V_{1/2}\right)/k\right]\right\}$$

where V is the condition pulse potential, $V_{1/2}$ is the potential at which the normalized I_{to} equals 0.5 and k is the slope factor. The estimates of $V_{1/2}$ and k were, respectively, −54 and 4.1 mV for control and −55.6 and 3.7 mV in the presence of 2 μM KC8851. Overall, the compound changed $V_{1/2}$ by 2.8 ± 0.4 mV (hyperpolarizing shift, $n = 5$) and k by 0.6 ± 0.2 mV ($n = 5$). The changes in these two parameters were not significant ($P > 0.05$). A similar lack of effect of KC8851 on the voltage-dependence of steady-state inactivation was also found (data not shown) at a concentration of 30 μM in five other cells.

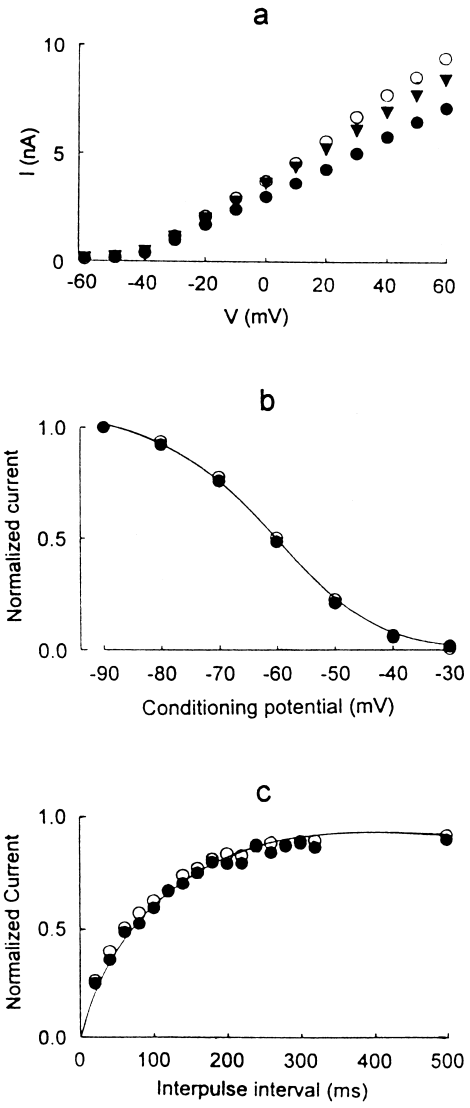

Fig. 3. Effects of KC8851 on properties of I_{to}. (a) Activation of I_{to} where peak currents are shown as a function of potential following depolarizing steps from a holding level of −70 mV. Control (open circles), KC8851 at 2 μM (closed circles) and after wash-off of KC8851 (closed triangles). (b) Actions of KC8851 on inactivation of I_{to}. Peak I_{to}, recorded using the protocol specified in the text, have been normalized and plotted vs. amplitude of the conditioning step. The solid line is a fit using the Boltzmann equation (see text). (c) Recovery of I_{to} from inactivation using a two-pulse protocol (see text). The solid line is an exponential fit to the data points with test peak I_{to} normalized by conditioning pulse peak I_{to}. The control data are shown as open circles and the effects of KC8851 at 2 μM are shown as closed circles. In (b) and (c), only the fits to control are shown.

The effects of KC8851 on the kinetics of recovery from inactivation were also determined. The procedure was to initially apply a depolarizing conditioning pulse to +60 mV for 200 ms to ensure full inactivation of I_{to}. This

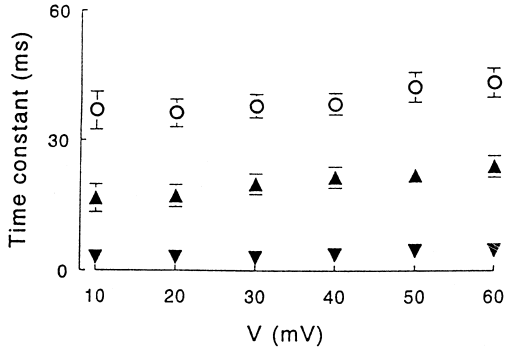

Fig. 4. Time constant (τ) dependence on voltage. The time constant (τ) values are means ± sem, with control (open circles), KC8851 at 2 μM (upward closed triangles) and 30 μM (downward closed triangles) ($n = 8$).

conditioning pulse was followed by a variable recovery time at −70 mV and a single test pulse of amplitude +60 mV to assess the extent of recovery. The recovery from inactivation was determined as the fractional change of I_{to} (test pulse peak current divided by condition pulse peak current). A typical result is shown in Fig. 3c where recovery from inactivation in the absence of drug (open circles) could be fit by an exponential with a time constant of 55 ms. In the presence of 2 μM KC8851 (Fig. 3c, closed circles), the time constant of recovery was 57 ms. In 4 other cells, studied with KC8851 at different concentrations (2–10 μM), there was no significant drug effect to alter the recovery from inactivation of I_{to} ($P > 0.05$).

3.4. Voltage dependence of decay time course and relation to channel block

The voltage-dependence of the inactivation time constant (τ) has been plotted in Fig. 4. The magnitudes of the time constants in control solution (open circles of Fig. 4) were not significantly altered ($P > 0.05$) by the different levels of voltage in the range of +10 to +60 mV. Also shown are the effects of KC8851, at concentrations of 2

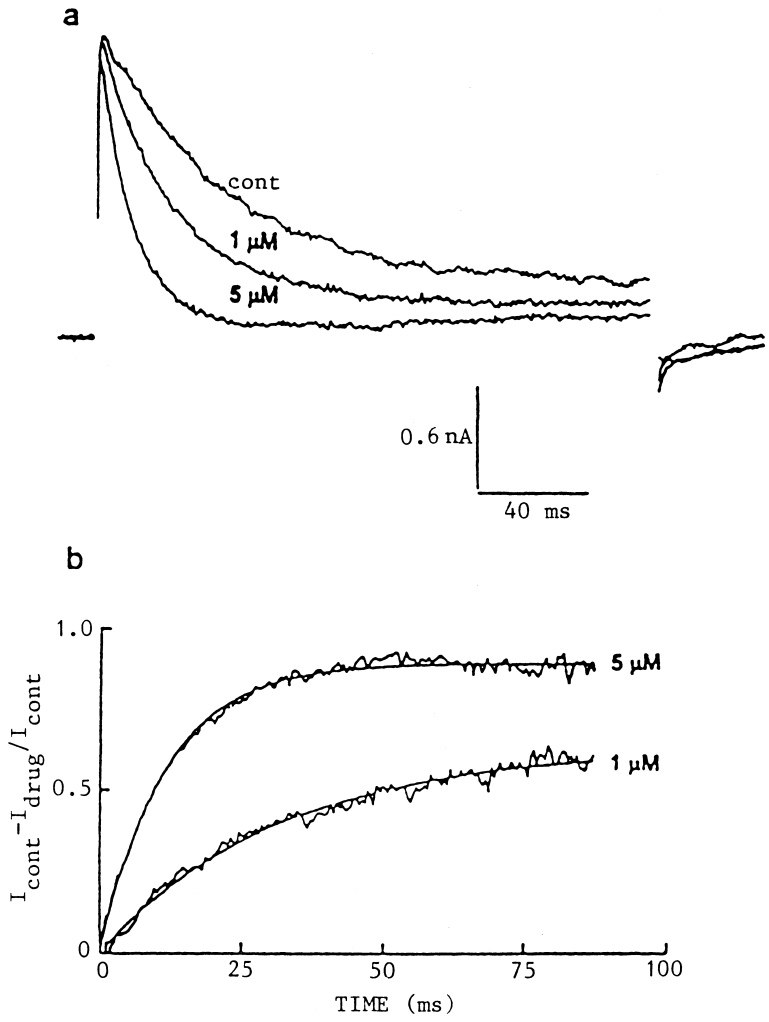

Fig. 5. Enhancement of channel blockade with time. (a) The traces shown are I_{to} in control (cont) and after addition of KC8851 at either 1 or 5 μM for a step depolarization to +60 mV. (b) The currents with KC8851 in (a) were first subtracted from control and then normalized by control. The fits shown were according to the equation in the text.

and 30 μM (upward and downward triangles of Fig. 4, respectively). The compound caused dose-dependent decreases in τ, however, for a given concentration there was no significant dependence of τ on potential ($P > 0.05$). Thus drug actions on the time course of decay were independent of voltage. These results would be consistent with a simple channel block model where the open state undergoes a transition to an open-blocked state with a voltage-independent rate constant k_1. In this case the inverse of the decay time constant in the presence of drug can be expressed as $k_1[D] + k_{-1}$ where D is the concentration of KC8851 and k_{-1} is the off or unblocking rate constant (McLarnon and Xu, 1995). An estimate for the blocking rate constant can be found by assuming that the rate constant $k_{-1} \ll k_1[D]$ and using the data from Fig. 4 to determine τ. For example, with KC8851 at a concentration of 30 μM, the k_1 was estimated to be 9×10^6 M^{-1} s^{-1} at +40 mV. Since the values of τ were relatively constant over the voltage range from +10 to +60 mV, the derived values of k_1 would not differ markedly from the value obtained at +40 mV. A more quantitative analysis for the channel block rate constants is considered below.

3.5. Rate constants for channel blockade using time-dependent inhibition of I_{to}

In order to also determine a value for the magnitude of the unblocking rate constant k_{-1}, measurements of the effects of two concentrations of KC8851 on the decay of I_{to} were carried out (Castle, 1990; McLarnon and Xu, 1995). In essence, the time course of KC8851 actions could then be determined by subtraction of the decay time course in the presence of the compound from that of control. Thus the development of enhanced inhibition of I_{to} with time was assumed to reflect a time-dependent block of open channels with rate constants k_1 (onward) and k_{-1} (off). This procedure was carried out at two different concentrations of drug and the magnitudes of k_1 and k_{-1} were determined using a solution of two simultaneous equations. Typical I_{to} in control (largest current) and with two concentrations of KC8851 (at 1 and 5 μM) are shown in Fig. 5a. The results of subtracting the two time-courses measured with drug from control data are presented in Fig. 5b where the results have been normalized using the expression $(1 - I_{drug}/I_{control})$. It can be noted that the increase of inhibition was both time and concentration-dependent. Secondly, the inhibition approached a maximum value which depended on the drug concentration. The following equation has been used to fit the curves shown in Fig. 5b:

$$I(t) = I_{max}(1 - e^{-(k1[D]+k-1)t})$$

where I_{max} equals maximum block at drug concentration $[D]$; $I(t)$ refers the amount of inhibition at any time t; and k_1 and k_{-1} are defined above. The single exponential fits, extrapolated to zero at the beginning of the depolarization steps, indicate that there was little block of I_{to} before activation. The analysis of the data shown in Fig. 5b gave $k_1 = 9.4 \times 10^6$ M^{-1} s^{-1} and $k_{-1} = 15.5$ s^{-1}. A similar analysis was also carried out on 3 other cells and the mean values of rate constants were: $k_1 = 9.3 \pm 0.6 \times 10^6$ M^{-1} s^{-1}, $k_{-1} = 19.3 \pm 6$ s^{-1}. The dissociation constant associated with channel block k_d ($k_d = k_{-1}/k_1$) was then found to be 2.1 μM, a value close to the EC_{50} for KC8851 effects on τ.

The possible use-dependence in KC8851 actions were also examined. A series of depolarizing steps to a level of +40 mV were applied from a holding potential of −70 mV. The stimulating frequency of pulses was set at 1 or 2 Hz so that control I_{to} showed no marked decrease of peak amplitudes. At concentrations of 2 and 10 μM, KC8851 diminished the amplitudes of currents and increased the decay of the current as noted previously. However, no evidence for use-dependence in KC8851 blockade of I_{to} was found.

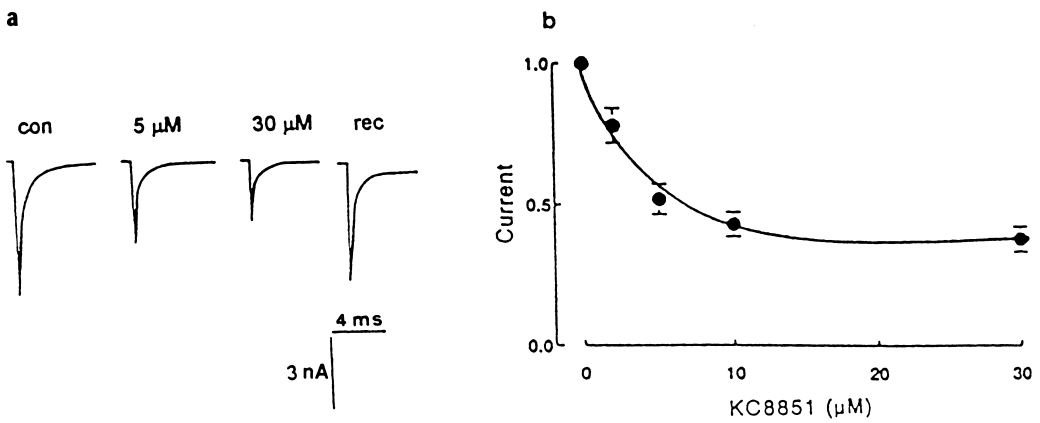

Fig. 6. Dose-dependent actions of KC8851 on I_{Na}. (a) Typical traces of I_{Na} in control (con), with KC8851 at 5 and 30 μM and after wash-off (rec). The protocols for recording I_{Na} are specified in the text. (b) Dose–response curve for normalized I_{Na}, with the fit shown using a decreasing exponential function, the data shown are means ± sem ($n = 9$).

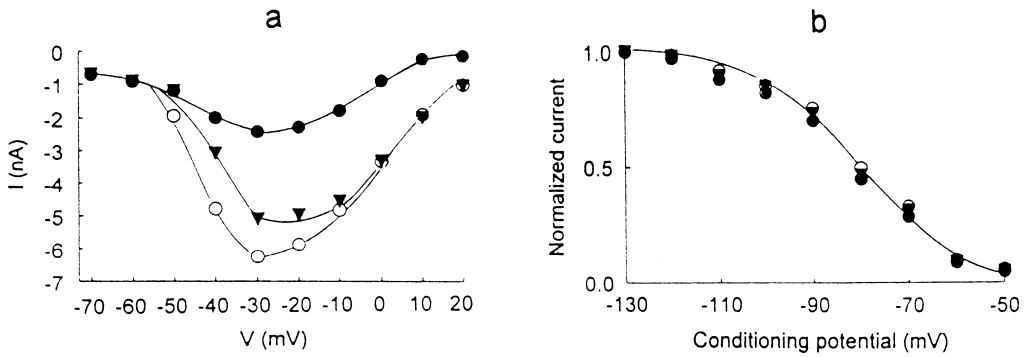

Fig. 7. Activation and inactivation of I_{Na}. (a) I/V relation showing activation of Na^+ currents. (b) Voltage dependence of the inactivation of I_{Na}. The solid line is a fit to control data; overall there was no significant effect of KC8851 to shift the voltage-dependence of inactivation. For both (a) and (b) control is presented as open circles, KC8851 (at 10 μM) as closed circles and wash-off as triangles.

3.6. Effects of KC8851 on I_{Na}

In vivo, the application of KC8851 leads to actions consistent with inhibition of Na^+ currents (McLarnon et al., 1996; Xu et al., 1997). Such actions were indicated from the increased magnitudes of PR intervals and also values of RSh after doses of KC8851 (0.125–4 μmol kg^{-1}) were applied. The nature of KC8851 interactions with I_{Na} was studied in vitro using a protocol which consisted of a prepulse to −130 mV, to remove inactivation present at the holding level of −70 mV, followed by a depolarizing test pulse to −10 mV.

Typical I_{Na} in control solution are illustrated and the effects of KC8851 are also shown at 5 and at 30 μM (Fig. 6a). The traces with KC8851 represent a steady level of block attained with the compound at a stimulation frequency of 10 Hz (the effects of different stimulation rates and use-dependent block by KC8851 are discussed below). The I_{Na}, following prolonged wash-off of KC8851, is shown as the final trace of Fig. 6a. The compound, in a

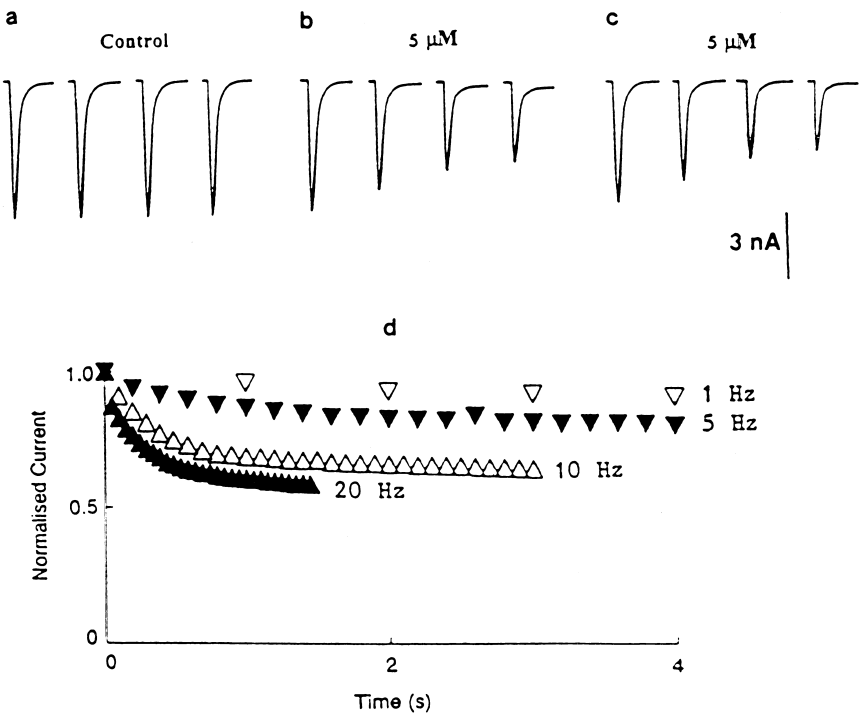

Fig. 8. Use-dependent inhibition of I_{Na}. (a) The traces shown are the I_{Na} for the pulses 1, 2, 6 and 30 of a 30 pulse sequence of depolarizing steps to −10 mV; frequency of 10 Hz. (b) Same protocol applied 2 min after the addition of KC8851 (5 μM) to the bath solution. (c) These traces were recorded 2 min after the last trace of (b) with the continued presence of KC8851 in the bath solution; same protocol as for (a). (d) Normalized I_{Na}, recorded with KC8851 (at 5 μM), the effects of different stimulation frequencies are shown.

dose-dependent manner, diminished the amplitude of the Na^+ current (mean peak amplitude in control was 6.4 ± 0.5 nA, $n = 9$) with no evident effects to alter the time course of inactivation. The overall results are presented as a dose–response plot where 4 concentrations of KC8851 were applied to each of the cells (Fig. 6b). The data were fit with a decreasing exponential function and the EC_{50} value derived from the dose–response plot was 5.1 ± 0.7 μM ($n = 9$).

3.7. Voltage dependence of activation and inactivation of I_{Na}

A typical I/V plot of the activation of I_{Na} is shown in Fig. 7a. The protocol utilized a series of depolarizing steps (range as shown in Fig. 7a) following an initial hyperpolarizing step to -130 mV. The voltage corresponding to peak Na^+ current was near -30 mV for control, KC8851 (10 μM), and following wash-off of the agent. Overall, in 7 cells there was no significant shift by 10 μM KC8851 (a value twice the EC_{50} for inhibition of current) in the voltage-dependent activation of I_{Na} ($p > 0.05$).

The voltage dependence of inactivation of I_{Na} was studied using a series of pre-pulses (range -130 to -40 mV) followed by a test potential to -20 mV. The results for one cell are presented in Fig. 7b and the data were fit using the same Boltzmann relation described above in the analysis of I_{to}. The value for $V_{1/2}$ was -84 mV in control and -87 mV with 10 μM KC8851. Inactivation relations were plotted for 7 cells and the effect of the compound was to shift the inactivation curve 3.1 ± 0.3 mV in a hyperpolarizing direction; this shift was not significant ($p > 0.05$).

3.8. Use-dependent actions of KC8851

The steady level of block by KC8851 (Fig. 6a) resulted from use-dependent inhibition of I_{Na}. The use-dependent blockade is illustrated in Fig. 8 where a frequency of stimulation at 10 Hz has been applied. The data shown are control (Fig. 8a) and after KC8851 (at 5 μM, Fig. 8b). As evident from Fig. 8a, no attenuation of currents was found in control. In this experiment the solution containing KC8851 was applied to the cell for two min prior to any stimulation. The first trace of Fig. 8b was attenuated slightly from that recorded in control, however, the subsequent I_{Na} showed a larger decrease to a steady level (traces shown correspond to stimulating pulses 1, 2, 6 and 30). Following a level of steady block, a period of another two min was allowed whereby no stimulation was applied. Despite the continued presence of the compound, the initial I_{Na} (first trace of Fig. 8c) evoked by a series of depolarizing pulses, was slightly reduced from control level and showed substantial recovery from the steady block. Thus KC8851 exhibited use-dependent blockade of I_{Na} with little evident tonic block (defined as block occurring in the absence of channel activation). Similar results were obtained from 4 other cells at concentrations of 5 μM KC8851; we did not investigate the effects of higher concentrations of the compound on the blockade of I_{Na}.

The effects of the different stimulation frequencies 1, 5, 10 and 20 Hz were studied in the presence of KC8851 (at 5 μM). As shown in Fig. 8d, at the lowest frequency there was only a small reduction in I_{Na} (normalized to the first current). With higher stimulations, the currents exhibited use-dependent blockade with the rate of inhibition increased at 10 and 20 Hz. A clear finding from the experiments was that the levels of inhibition of I_{Na} reached steady levels at each frequency with the largest degree of inhibition obtained at 20 Hz. The same procedures were applied to 4 other cells (only 5 μM was used in these studies) with essentially identical results to those presented in Fig. 8d.

3.9. Effects of KC8851 on other cardiac currents I_{K1} and I_{Ca}

The overall selectivity of a compound for effects on particular channels is an important factor in describing potential therapeutic efficacy. In rat ventricle both inward rectifier K^+ currents and inward Ca^{2+} currents contribute to the genesis of the cardiac action potential. In these experiments the single objective was to determine if a single concentration of KC8851 (at 20 μM) had any effect on I_{K1} or I_{Ca}. This concentration was chosen as a value well above the EC_{50} for blockade of I_{to} and I_{Na}. The I_{K1} was activated with hyperpolarization steps from a resting potential of -60 mV to levels up to -140 mV. At 20 μM KC8851, no significant change in amplitudes or time courses were found ($P > 0.05$, $n = 6$). Inward I_{Ca} were evoked with depolarizing steps above -10 mV (holding potential of -60 mV) and could be recorded for up to 6 min before a slow run-down of amplitude ensued. Measurements were obtained within 30 s after attaining the whole-cell clamp and in no case ($n = 5$) was there any change noted in the amplitudes or time courses of currents. Thus KC8851, at a concentration of 20 μM, had no significant actions to alter either I_{K1} or I_{Ca}.

4. Discussion

The most significant finding arising from this study was that KC8851, at relatively low concentrations, exhibits blockade of both I_{to} and I_{Na}. This result is consistent with findings from in vivo studies in rats (McLarnon et al., 1996, Xu et al., 1997) where KC8851 widened the K^+-dependent variable, Q–T interval (D_{25} of 0.16 μmol kg^{-1} min^{-1}) and increased the Na^+-sensitive quantities, PR interval (D_{25} of 2.3 mol kg^{-1} min^{-1}) and RSh (D_{25} of 0.2 μmol kg^{-1} min^{-1}). This mixed blockade of the currents I_{to} and I_{Na} is different from that found with the structural

analogue of KC8851, tedisamil, a bradycardic agent (Oexle et al., 1987; Wallace et al., 1995). Tedisamil has been shown to be a potent blocker of I_{to}, but with little or no inhibition of I_{Na} at concentrations less than 20 μM (Dukes et al., 1990). Furthermore, in vivo studies in rats (Beatch et al., 1991; Adaikan et al., 1992) have also indicated minimal effects of tedisamil on Na^+ currents. Thus both in vitro and in vivo KC8851, but not tedisamil, exhibits a mixed profile of K^+ and Na^+ blockade in rat ventricle.

The studies have clarified mechanisms of actions of KC8851 on different states of I_{to}. The compound had no effect on any aspects involving the inactivated state including the voltage-dependence of inactivation and the time course of recovery from inactivation. Instead, the effects of KC8851 to hasten decay of I_{to} in a dose-related fashion (Fig. 2d) and to produce an enhanced effect with time after channel activation (Fig. 5b) strongly suggested interactions with the open state. A sequential blockade model was applied to yield mean values of rate constants for channel block of 9.3×10^6 M^{-1} s^{-1} and unblock of 19.3 s^{-1}, giving an estimated k_d of 2.1 μM. The magnitudes of the net onward rate constant $k_1[D]$ are sufficiently large (e.g., near 50 s^{-1} at 5 μM) such that some decrease in peak amplitude of I_{to} would be expected during the activation phase. In this case some I_{to} channels would be blocked as I_{to} became fully activated over a period of 5 ms and peak amplitude would be reduced.

The present experiments do not allow for a clear distinction of the diminished peak I_{to} as due to the magnitudes of the blocking rate constant or the possibility of some tonic block by KC8851. The magnitude of the k_d for KC8851 block of I_{to} is low, as a comparison the following values have been obtained for other compounds; tedisamil (2–5 μM) (Dukes et al., 1990), bupivacaine (23 μM) (Castle, 1990), octylacaine (3.5 μM) (Castle, 1990) and terikalant (17 μM) (McLarnon and Xu, 1995). The unblocking rate constant for tedisamil has been estimated at 25 s^{-1} (Dukes et al., 1990) which is close to the value derived in the present studies for KC8851. Thus KC8851 and tedisamil show no evident differences in the rate constants for open channel blockade of I_{to}. The larger k_d for bupivacaine and terikalant primarily reflect higher values of k_{-1} which could limit the effectiveness of channel blockade by the agents. It has been suggested that the magnitudes of k_{-1} associated with block of I_{to} are correlated with the degree of hydrophobicity of the agent (Castle, 1990). The relatively long times for the recovery of KC8851 block of I_{to} would indicate actions of the compound from inside; previous work has suggested that tedisamil actions on this current were consistent with block at an internal site (Dukes et al., 1990).

The actions of KC8851 to inhibit I_{Na} were use-dependent with effects enhanced at higher rates of channel activation. This result would be consistent with actions of the compound on the open state of the Na^+ channel. As noted previously, this property would be expected to have utility in the prevention of arrhythmic activity (Tamargo et al., 1992). In terms of state-dependency, the results would indicate that KC8851 had no significant effects on the activation or inactivation states of the Na^+ channel. For example, the compound had minimal effects to shift the voltage-dependence of channel inactivation. However, there was some tonic block of I_{Na} as reflected by a decrease in current amplitude at the start of the stimulation series (Fig. 8b, c) suggesting possible small effects on the closed state. This result would indicate that KC8851 may interact with more than the open state of the system.

At concentrations below 20 μM, KC8851 had little or no effects on I_{K1} or I_{ca}. Although we did not quantitate effects of KC8851 on any other currents, it is evident from the data shown in Fig. 2 that the compound, at concentrations as low as 2 μM, diminished the steady state current remaining after the transient I_{to} had inactivated. This residual current has different pharmacological and kinetic properties from I_{to} and may represent activation of a delayed rectifier K^+ channel (Apkon and Nerbonne, 1991). Previous work has documented inhibition of delayed rectifier K^+ currents by tedisamil in rat heart (Dukes et al., 1990) and guinea pig portal vein (Pfrunder and Kreye, 1992).

In summary, KC8851 has been found to be a potent mixed blocker of I_{to} and I_{Na} whereas the analogue, tedisamil, acts only on I_{to} at low concentrations. Neither compound exhibits effects on I_{K1} or I_{Ca} at a concentration of 20 μM. This result has relevance in applying structure–activity data to the question concerning a possible correlation between blockade of specific channels with the in vivo efficacy of putative antiarrhythmic agents. Thus, an in vivo comparison of the antiarrhythmic efficacy of a selective K^+ blocker (tedisamil) with that of a mixed K^+/Na^+ blocker (KC8851) would be expected to have utility as to the role of structure in the development of novel antiarrhythmic compounds.

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