0026-895X/91/040540-07$03.00/0

MOLECULAR PHARMACOLOGY, 39:540-546

Actions of Cardiac Drugs on a Calcium-Dependent Potassium Channel in Hippocampal Neurons

JAMES G. MCLARNON and XUE-PING WANG

Department of Pharmacology and Therapeutics, Faculty of Medicine, The University of British Columbia, Vancouver, British Columbia, V6T 123, Canada

Received September 24, 1990; Accepted January 22, 1991

SUMMARY

The patch-clamp method has been used to determine the actions of three newly synthesized cardiac drugs on a calcium-dependent potassium channel, K(Ca), in CA1 hippocampal neurons. Activation of a 65-pS channel was evident in excised inside-out patches with the internal side of the membrane exposed to Ca^{2+} (0.2 mM); cessation of channel activity was immediate upon perfusion with ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid-containing solution. In the presence of low concentrations (0.1–10 μM) of the drugs UK-68798, KC-8857 (tedisamil), or WY-48986 (risotilide), channel openings evinced rapid flickering behavior from open to nonconducting levels; current amplitude was not affected by the drugs. The actions of the drugs were consistent with a voltage-independent block of open K(Ca) channels. In addition, the three drugs, at concentrations similar to those applied to inside-out patches, also blocked K(Ca) when they were applied to the bath solution for outside-out patches. The potencies for channel block of the drugs acting either externally or internally were in the order UK-68798 > tedisamil > risotilide, with UK-68798 reducing the mean open time of K(Ca) by one-half at a concentration near 0.4 μM.

Considerable efforts are presently being directed towards the development of improved drugs that possess actions on specific ion channels in cardiac tissue. For example, drugs that would act selectively on sodium, calcium, or potassium channels in cardiac cells could have potential therapeutic use as antianginal or antiarrhythmic agents. In addition to establishing specificity and mechanisms of actions for the drugs on ion channels in cardiac cell membrane, it is also important to determine whether such agents act, at low concentrations, on noncardiac tissue. Recent work (1) has shown that the drug KC-8851 blocks a K(Ca) in hippocampal neurons at concentrations similar to those at which a chemically related agent, KC-8857 (tedisamil), increases the inactivation rate of the transient outward potassium current in rat ventricular myocytes (2). Of interest is the observation that tedisamil, at a dose twice that producing maximum antiarrhythmic actions in rat, also caused adverse effects on respiration that could be a consequence of drug activity in the central nervous system (3). In the present work, the actions of three newly synthesized cardiac drugs, UK-68798, KC-8857 (tedisamil), and WY-48986 (risotilide), have been studied on K(Ca) in CA1 hippocampal neurons. All of these agents evinced actions at relatively low concentrations, which were consistent with channel block of K(Ca). The potency, as defined by the ability to decrease channel mean open time, varied markedly between the agents.

J.G.M. is the recipient of a research scholar award and an operating grant from the British Columbia Health Care Research Foundation.

Experimental Procedures

Cell cultures. The CA1 hippocampal neurons were isolated from 18-day embryonic-age rat fetuses and cultured following procedures described previously (4). After dissection, the hippocampi were subjected to enzymatic and mechanical treatment to dissociate the cells. The cell count was adjusted with Dulbecco's modified Eagle's medium, and laminin-coated coverslips, treated with poly-D-lysine, were plated with a cell density of approximately 10^5 cells/cm^2. The coverslips were then incubated, with the growth side downwards, in Dulbecco's modified Eagle's medium with 5% CO_2 at 37°. After 3–5 days, the cells were treated with 5-fluorodeoxyuridine to prevent glial cell multiplication. The cells were maintained in culture solution, and the growth medium was half-changed every 3 days.

Electrophysiology. The electrophysiological recordings were carried out between days 5 and 15 after cell isolation. Single-channel recordings were taken from excised patches for both inside-out and outside-out patch-clamp modes. With the inside-out configuration, the pipette solution contained 140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES, pH 7.3. The bath solution used before and during patch excision had the same composition as the pipette

ABBREVIATIONS: K(Ca), calcium-dependent potassium channel; $I_K(Ca)$, Ca^{2+}-activated potassium current; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; HEPES, *N*-2-hydroxyethyl-piperazine-*N′*-2-ethanesulfonic acid; TEA, tetraethylammonium chloride; tedisamil, 3,7-di(cyclopropylmethyl)-9,9-tetramethylene-3,7-diazabicyclo-(3.3.1)nonane dihydrochloride (KC-8857); I_{TO}, transient outward potassium current; I_K, delayed rectifier potassium current.

solution, with the exception that the Ca^{2+} concentration was 0.2 mM. After patch excision, the bath solution was exchanged to one containing high K^+ (140 mM KCl, 5 mM NaCl, other ions unchanged). In some experiments, the bath concentrations of Ca^{2+} were lowered either to 0.02 mM or to values below 0.1 μM, with the addition of EGTA (at concentrations of 0.19 and 1 mM, respectively) to the 0.2 mM Ca^{2+} solution; the concentrations of EGTA were determined from published data (5, 6). For outside-out patches, the bathing solution had 140 mM NaCl, 5 mM KCl, and 1 mM $CaCl_2$ (other ions at the same concentration as for the standard bath solution for inside-out patches). The pipette contained 140 mM KCl, 5 mM NaCl, and 0.2 mM $CaCl_2$ (other ions at the same concentrations as for the pipette solution described above). The drugs were added to the bath solutions for perfusion of the inside-out and outside-out patches and included tedisamil (KC-8857, Kali-Chemie; Hannover, Germany), UK-68798 (Pfizer; Kent, England), and risotilide (WY-48986; Wyeth-Ayerst, Princeton, NJ). Risotilide and UK-68798 are sulfonamide compounds (7), and tedisamil is a heterocyclic dihydrochloride compound (8) that is based on sparteine (structures of the agents are shown in Fig. 1).

The single-channel currents were recorded using an Axopatch amplifier (Axon Instruments), with the current filtered (Bessel characteristic, four-pole) at either 2 kHz or 5 kHz. Data were digitized at sampling rates of 5 kHz (low-pass filter set at 2 kHz) or 20 kHz (low-pass filter set at 5 kHz). The latter setting was used for recording rapid channel flickering events between open and nonconducting states, which were characteristic with application of higher concentrations of the drugs. The analysis routines used pClamp (version 5.0 or 5.5) to determine distributions for channel amplitudes, open times, and closed times. A minimum of 300 events, assembled from records containing only a single level of opening, were used to define the distributions. All data were obtained at room temperature (22–24°).

Results

Inside-out patches. Records from inside-out patches (V = 0 mV) with 5 mM K^+ and 0.2 mM Ca^{2+} in the bathing solution did not show channel activity (Fig. 2a); in this case, the K^+ concentrations across the patch are equal. Upon perfusion of the inside-out patches with a bath (control) solution containing 140 mM K^+ and 0.2 mM Ca^{2+}, activation of a large conductance channel was apparent (Fig. 2b). Introduction of bath solution with 140 mM K^+ and EGTA (1 mM) resulted in abrupt cessation of channel openings (Fig. 2c), with reperfusion of control solution establishing channel activity (Fig. 2d). These results are, thus, similar to those described previously for the activation of a K(Ca) in cultured hippocampal neurons (1). In the latter work, exchange of the high K^+ bath solution (140 mM) for one containing 70 mM K^+ and 70 mM Na^+ caused a depolarizing shift in the zero-current potential, which was consistent with the predictions of the Nernst relation with channel selectivity for K^+. The current-voltage relation from the cumulative analysis of eight inside-out patches (including the patch activity illustrated in Fig. 2) is shown in Fig. 3. The slope conductance of the K(Ca) was determined to be 65 pS, with an extrapolated zero-current potential of −70 mV [respective values of 62 pS and −72 mV were determined in the previous study (1)].

Calcium-dependence of K(Ca). As noted previously, when the free Ca^{2+} was lowered to values below 0.1 μM, the K(Ca) was quiescent. The kinetics of K(Ca) were also studied with 0.02 mM Ca^{2+} included in the solution bathing inside-out patches. Decreasing the Ca^{2+} concentration from 0.2 mM to 0.02 mM diminished both the mean open times and the frequency of channel openings. The mean open time was decreased by 62 ± 8% (n = 4 patches), and the frequency of channel events was decreased by 71 ± 16% (n = 4 patches) with the 10-fold decrease in Ca^{2+}. The net effect on the kinetics of K(Ca) when the Ca^{2+} concentration was decreased by a factor of 10 would be to lower the probability of channel opening by about 90%.

Voltage dependence of the mean open time for K(Ca). At a patch potential of 0 mV, the mean channel open time with 0.2 mM Ca^{2+} in the bathing solution was 14.7 ± 1.0 msec (n = 11). When the patch potential was changed to 20 mV, which increased the driving force for outward K^+, the mean open time was 15.3 ± 1.1 msec (n = 11). In three of the patches, the mean open times were measured over a wider range of patch potentials; the values of open times at V = 60 mV were not significantly different from those determined at V = 0 mV. Thus, the mean open time for K(Ca) was not dependent on potential.

Effects of drugs on K(Ca). The effects on I_K(Ca) of the drugs UK-68798, tedisamil, and risotilide were studied by addition of the agents to the 140 mM K^+ solution bathing the inside of the cell. Openings in the absence of the drug are shown in Fig. 4a, and the addition of UK-68798 (at 0.1 μM; Fig. 4b) resulted in an increase in the frequency of transitions from the open level to a nonconducting state, with no change in the amplitude of the currents. Increasing the concentration of UK-68798 (to 0.5 μM) caused a further increase in flickering between open and nonconducting states, as shown in Fig. 4c. In order to resolve the transitions between levels, the sampling rate was increased to 20 kHz and the low-pass filter was set at 5 kHz (Fig. 4d). Typical distributions for the amplitudes and the open and closed times are shown in Fig. 5, a, b, and c, for channel activity in the absence and in Fig. 5, d, e, and f, for activity in the presence of UK-68798. The amplitude distributions were not changed by the drug. The distributions for closed times in control solution were best fit with two components, reflecting the bursting activity of the channel. The fast component of the closed distributions was due to gaps within bursts, and the longer component represented the closed times between bursts. UK-68798 had no significant effect to alter either of the two components in the distributions of closed times. The essential action of the drug was to decrease the open time of the channel by causing rapid flickering between the open state and a pre-

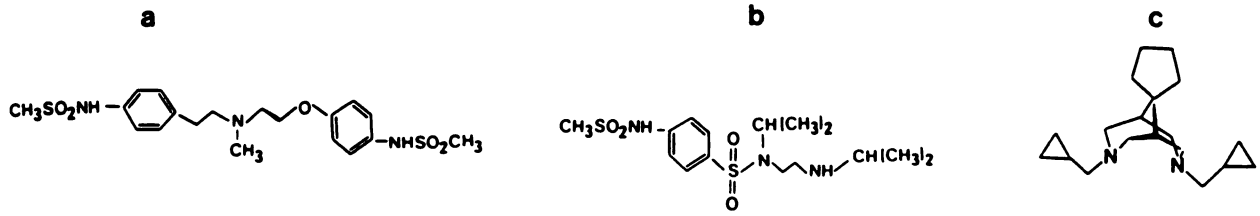

Fig. 1. Chemical structures of drugs used in the experiments. a, UK-68798; b, risotilide; c, tedisamil.

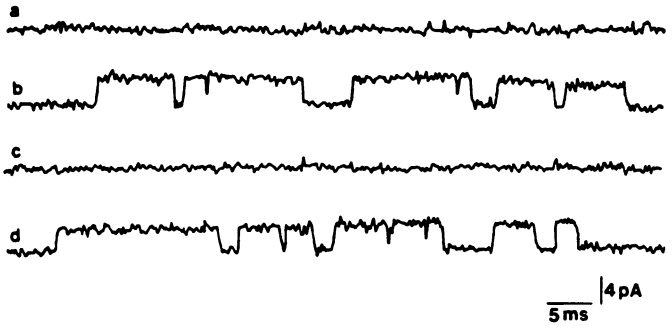

Fig. 2. Unitary currents through $I_K(Ca)$ with $V = 0$ mV; inside-out patch. a, Bath solution containing 5 mM K^+. b, Bath solution (control) containing 140 mM K^+; channel openings are upward from the baseline. c, Control bath solution with EGTA (1 mM). d, Return to control solution. In all solutions, the Ca^{2+} was 0.2 mM. For all records, the low-pass filter was set at 2 kHz.

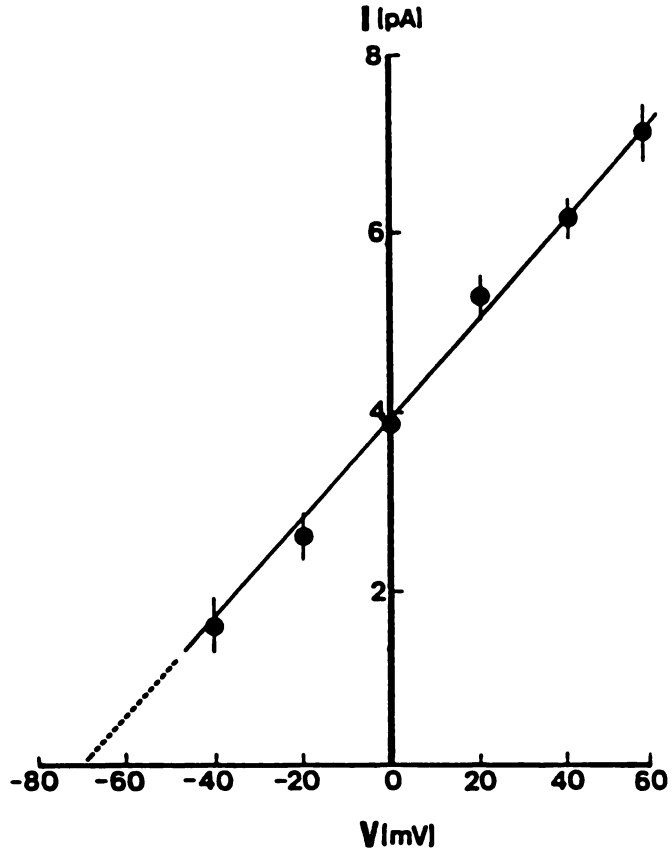

Fig. 3. Current-voltage plot for K(Ca). The *data points* are mean values from eight inside-out patches in control solution and the *error bars* are standard error values. The slope conductance was 65 pS and the zero-current potential was −70 mV.

sumed blocked state. All distributions for channel open times, in the absence or the presence of UK-68798, were well fit with single-exponential functions. When the driving force was increased by changing the patch potential from 0 mV to 20 mV or 40 mV, the channel amplitudes were increased, with little or no change in the frequency of transitions from open to blocked states (Fig. 6). For four inside-out patches with 0.2 μM UK-68798 present in the bath solution, the ratio of open time at $V = 0$ mV to that at $V = 20$ mV was determined to be 0.92 ± 0.12; thus, the blocking action of UK-68798 showed no significant dependence on patch potential.

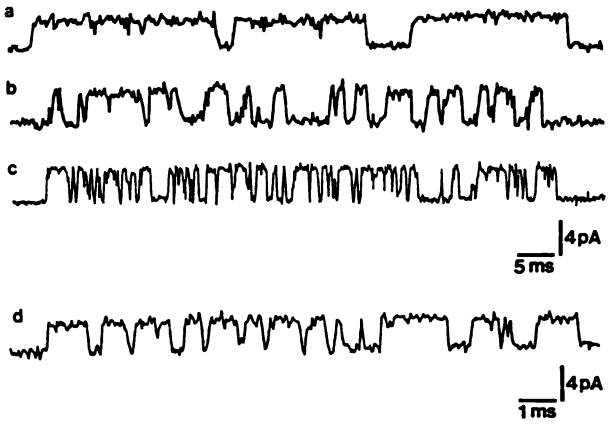

Fig. 4. Unitary currents (upward deflections) with $V = 0$ mV and inside-out patch. a, Openings in control (140 mM K^+) bath solution. b, Openings with UK-68798 (0.1 μM). c, Openings with UK-68798 (0.5 μM). In a–c the sampling frequency was 5 kHz and the low-pass filter was set at 2 kHz. d, Openings with UK-68798 (0.5 μM), with sampling frequency increased to 20 kHz and low-pass filter at 5 kHz.

The effects of tedisamil and risotilide on the single-channel properties of K(Ca) were qualitatively similar to those seen with UK-68798; however, higher concentrations of the drugs were required to produce channel block. The actions of risotilide (at 5 μM) and tedisamil (at 1 and 5 μM) are shown in Fig. 7. The amplitude and closed time distributions in the absence of the drugs were not significantly changed when tedisamil or risotilide was applied to inside-out patches. Channel mean open times were significantly decreased by the drugs; with tedisamil (at 1 μM) the open times were decreased by 52% ($n = 5$ patches), and with risotilide (at 5 μM) the open times were decreased by 38% ($n = 3$ patches). The drug actions to decrease open times were not significantly changed when the patch potential was changed to 20 mV. The ratio of open times at $V = 0$ mV to that at $V = 20$ mV was 1.03 ($n = 5$; tedisamil at 1 μM) and 0.96 ($n = 3$; risotilide at 10 μM).

Several different concentrations for each of the drugs, UK-68798, tedisamil, and risotilide, were applied to inside-out patches. The results of the drug actions on the mean channel open time are summarized in Table 1 and show that the potencies of the drugs to attenuate the channel open times varied markedly and were in the order (from highest to lowest) UK-68798, tedisamil, and risotilide.

The increased channel flickering and consequent reduction in mean open time observed with increasing concentrations of the drugs is similar to that found previously (1) for the actions of KC-8851 (structurally related to tedisamil) on $I_K(Ca)$. The actions of the drugs to diminish mean open times, coupled with no significant changes in the amplitudes of single-channel currents, are consistent with open channel blockade of K(Ca). A sequential scheme in which open channels can be blocked by drug molecules can be represented in equation form as $\tau^{-1} = k_1 + k_2[D]$, where τ is the channel mean open time in the presence of the drug, k_1 is the normal channel closing rate constant, k_2 is the blocking rate constant, and $[D]$ is the drug concentration. In essence, the mean channel open time in the presence of the drug is reduced, because the open state can reach a blocked state via the k_2 pathway, in addition to the normal transition to the closed level. The magnitudes of k_2 were determined by

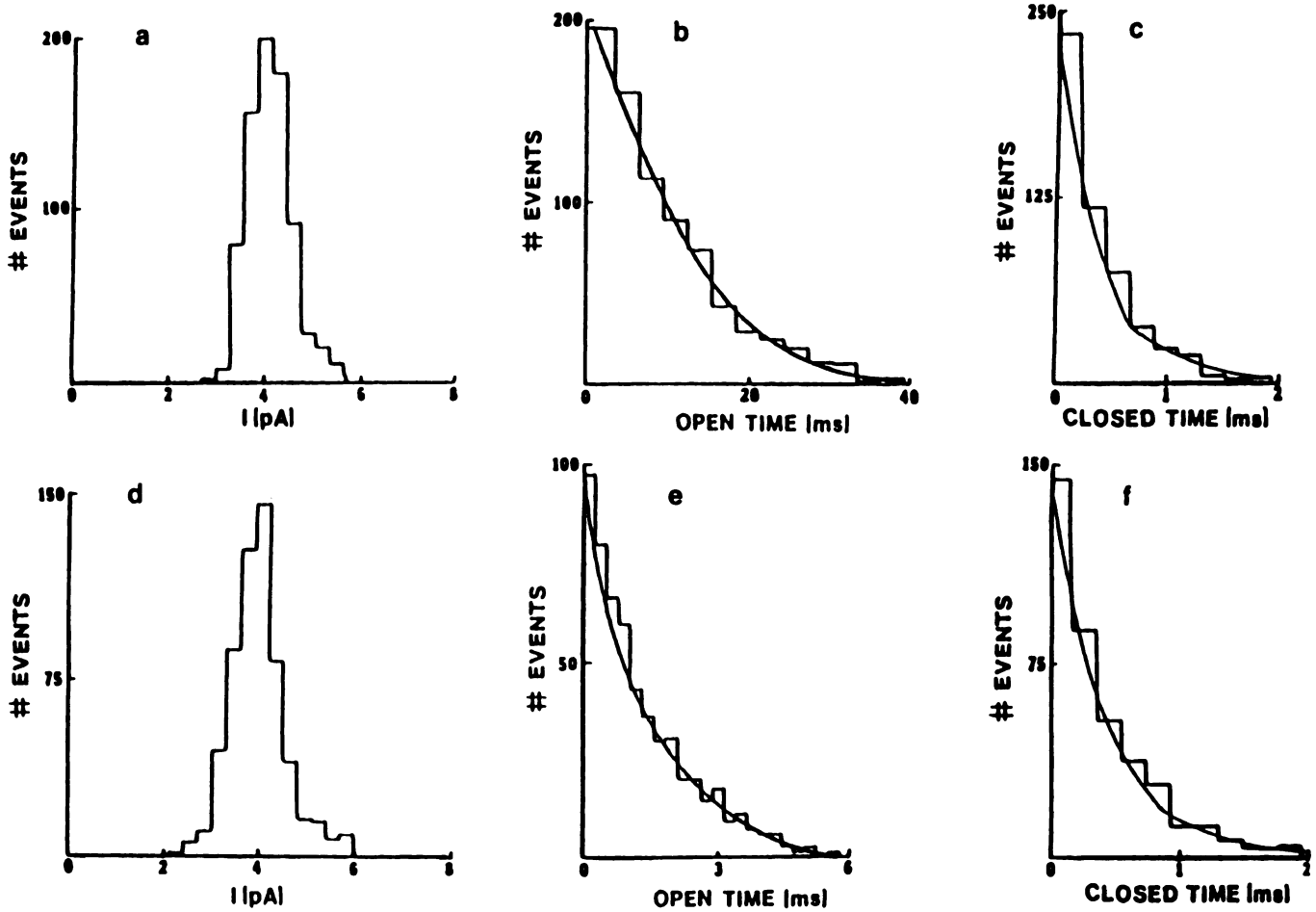

Fig. 5. Amplitude and time distributions. a–c, Distributions with control solution for amplitude (4.1 pA) (a), open time (12.5 ± 0.6 msec) (b), and closed time (two components with values of 0.9 ± 0.4 and 1.8 ± 0.2 msec) (c); a total of 950 events were recorded for each distribution. d–f, Distributions with UK-68798 (1 μM) for amplitude (4.0 pA) (d), open time (1.4 ± 0.3 msec) (e), and closed time (two components with values of 0.9 ± 0.4 and 2.0 ± 0.4 msec) (f); a total of 625 events were recorded for each distribution.

Fig. 6. Unitary currents, in the presence of UK-68798 (0.5 μM), at different patch potentials; inside-out patch. a, $V = 0$ mV; b, $V = 20$ mV; c, $V = 40$ mV.

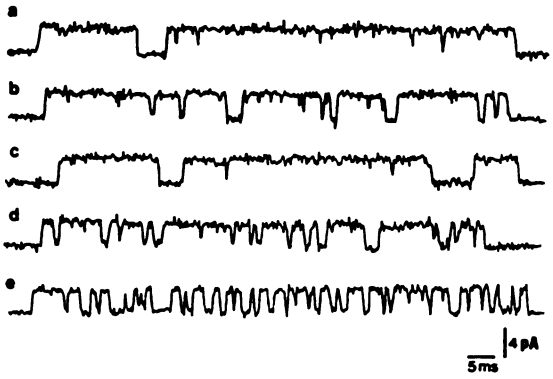

Fig. 7. Unitary currents with risotilide and tedisamil at $V = 0$ mV; inside-out patch. a, Control solution; b, risotilide (5 μM) added to control solution; c, re-control solution; d, tedisamil (1 μM) added to control solution; e, tedisamil (5 μM) added to control solution. The low-pass filter was set at 2 kHz.

first finding k_1 in the absence of the drug and using the values for mean open time with the drug present. The k_2 values are included in Table 1 and show that UK-68798 had the highest potency, as determined from the magnitudes of k_2, for channel block, followed by tedisamil and then risotilide. The values of k_2 were not significantly changed when $V = 20$ mV or $V = 40$ mV; thus, the blocking actions of the drugs were not dependent on patch potential. The approximate values for the drug concentrations required to halve the mean open time were as follows: UK-68798, 0.4 μM; tedisamil, 1 μM; and risotilide, 7.5 μM.

Outside-out patches. The actions of the three drugs were also studied by addition of the agents to the solution bathing

TABLE 1

Normalized mean open times and onward (blocking) rate constants for drugs

The normalized mean open times, at $V = 0$ mV, were determined by dividing the open times in the presence of the drugs by the mean open times in control solution (14.7 ± 1.0 msec, $n = 11$, inside-out mode; or 17.7 ± 1.8 msec, $n = 9$, outside-out mode). The values for k_2 were then determined using the simple open-channel block scheme.

Drug	Concentration	n	Mean open time	k_2
	μM			$10^7\ M^{-1}\ sec^{-1}$
Inside-out mode				
UK-68798	0.1	3	0.71	24.5
	0.5	4	0.40	17.7
	1.0	2	0.19	25.1
Tedisamil	1.0	5	0.48	6.4
	5.0	4	0.16	6.2
	10.0	3	0.11	5.3
Risotilide	5.0	3	0.62	0.7
	10.0	2	0.35	1.1
Outside-out mode				
UK-68798	0.2	3	0.52	25.4
	0.5	5	0.34	21.3
	1.0	3	0.20	22.0
Tedisamil	2.0	4	0.24	8.7
Risotilide	5.0	3	0.50	1.1
	10.0	3	0.35	1.2

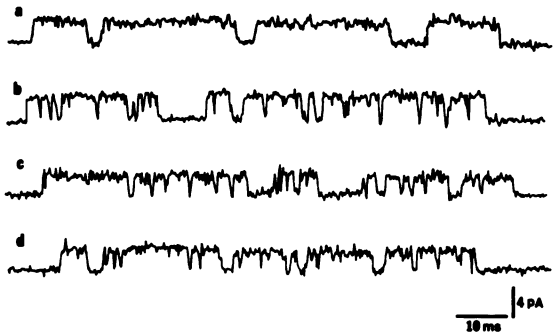

Fig. 8. Unitary currents (upward openings) in the absence and presence of the drugs, at $V = 0$ mV; outside-out patch. a, Control bath solution (containing 5 mM K^+ with 140 mM K^+ in the pipette solution); b, with UK-68798 (0.1 μM) added to control; c, with tedisamil (1 μM) added to control; d, with risotilide (5 μM) added to control. All data are from the same patch, with re-control solution applied between each drug application (not shown); low-pass filter was set at 2 kHz.

outside-out patches. In these experiments, the pipette solution contained 140 mM K^+ and 0.2 mM Ca^{2+} and the bath solution contained 5 mM K^+ and 1.0 mM Ca^{2+}. Typical channel openings, with the patch potential held at 0 mV, are shown in Fig. 8a. The application of the agents to the outside-out patch caused rapid flickering transitions to a nonconducting state (Fig. 8, b, c, and d), in a manner similar to that found with inside-out patches. All of the drugs had significant effects to decrease the mean open time of K(Ca) (Table 1). The channel open times were lower with increasing concentrations of UK-68798 or risotilide; only one concentration of tedisamil was applied to outside-out patches.

In some experiments, the voltage dependence of channel mean open time was measured for both control solutions and solutions with the drugs. The mean open time in control solution was found to be 17.7 ± 1.8 msec, from analysis of nine patches. This value was not significantly changed when $V = 20$ mV (ratio of mean open time at $V = 0$ mV to V = 20 mV was 1.11 ± 0.2; $n = 6$ patches); thus, the channel mean open time was not dependent on patch potential. In addition, the drug actions to diminish open time were not affected when the patch potential was changed to 20 mV. The ratios of open times at 0 mV to those at 20 mV for the different drugs were as follows: UK-68798, 0.95 ± 0.06 ($n = 6$); tedisamil, 0.90 ± 0.08 ($n = 4$); and risotilide, 0.93 ± 0.08 ($n = 3$). These data include groupings of patches for different concentrations of UK-68798 (0.5 and 1.0 μM) and risotilide (5 and 10 μM); only one concentration of tedisamil was used (2 μM). The distributions for channel amplitudes and closed times were not significantly changed with any of the concentrations of the drugs.

Analysis of the data obtained from outside-out patches was done using the simple sequential open-channel block scheme, and values for the blocking rate constant k_2 are included in Table 1. The potency for channel block, as defined by the magnitudes of k_2, for external application of the drugs was in the order UK-68798 > tedisamil > risotilide, that is, the same as determined for inside-out patches. Because the mean open times for K(Ca) were not altered, in both the absence and the presence of the drugs, the blocking rate constant k_2 was not voltage dependent. The voltage dependence of k_2 (or mean open time) was not systematically investigated over a wider voltage range; however, in experiments where potentials of 40 mV and 60 mV were applied to outside-out patches, no obvious changes in flickering activity were apparent, compared with the patterns at the lower patch potentials. The approximate drug concentrations causing a 2-fold decrease in channel mean open time were 0.2 μM for UK-68798 and 5 μM for risotilide; although only one concentration of tedisamil was used, a value close to that found with inside-out patches (1 μM) was suggested from the data.

Discussion

Single-channel currents through K(Ca) in cultured hippocampal CA1 neurons are sensitive to the actions of drugs that fit the general description of class III antiarrhythmic agents (2, 7). The data are consistent with open-channel blockade as the mechanism of action for UK-68798, tedisamil, and risotilide on I_K(Ca); similar results were found for the effects of KC-8851 (structurally related to tedisamil) in previous work (1). The amplitudes of the single-channel currents were not significantly altered by any of the drugs, and channel openings in the presence of the agents showed increased numbers of transitions from the open level to a nonconducting state. The frequency of the transitions was increased with the dose of the drug. A simple open-channel sequential scheme was used to determine the onward (blocking) rate constants (k_2) for the drugs, and the potency of the agents to diminish channel mean open time, for both external and internal patch applications, was in the order UK-68798 > tedisamil > risotilide. Although the majority of experiments were carried out at $V = 0$ mV, additional studies were done at other patch potentials. In particular, the mean open times of the K(Ca) channel were not significantly changed with the drug applications to inside-out patches (with $V = 20$ mV) or to outside-out patches (with $V = 20$ mV). These results would suggest that the neutral species of the drugs were the

active forms and, furthermore, that hydrophobic membrane sites were involved in the drug actions. A hydrophobic binding site is also consistent with the result that UK-68798 has a considerably higher potency for channel block than does risotilide. The two agents have similar structures; however, UK-68798 would have a higher degree of lipid solubility than would risotilide. Tedisamil, which is not a sulfonamide compound, exhibited a blocking potency that was intermediate between those of UK-68798 and risotilide. It is possible that tedisamil may be acting at a different site than the other drugs or at the same nonspecific hydrophobic site. The similarities in the k_2 values for the drug actions on inside-out or outside-out patches would suggest that the blocking site(s) are mainly accessible from one side of the membrane. For example, if the binding site was reached from the internal side of the membrane or from the lipid, then external applications of the drugs could block the channel subsequent to diffusion of the agents through the lipid region.

A detailed analysis for the off-rate constant (k_{-2} in the simple open-channel block scheme) was not possible. In the absence of the drugs, the single-channel records showed bursting behavior of channel activity, and two-component exponential functions were generally required to fit distributions of closed times. In most cases, the magnitudes of the two components were similar, and on the basis of the single-channel records it was difficult to distinguish between shut and blocked states. In essence, bursts of channel-blocking events were often not resolvable from sojourns to the shut level (such as occurred in the absence of the drug) in the records. Estimates for the dissociation constant, K_D, could be obtained from the single-channel records, however, if one assumed that in the presence of the drug the majority of rapid transitions from the open state represent blocking episodes. Thus, for example, in the records for UK-68798 (Fig. 4d) the off-rate constant was estimated to be near 1000/sec, which, coupled with the blocking rate constant from Table 1, would yield a value for K_D near 5 μM.

All of the drugs studied here are generally classified as class III antiarrhythmic agents, by virtue of actions to prolong action potentials in cardiac tissue with little or no effect to slow conduction velocity; such actions are consistent with block of K^+ channels (7). This work includes studies of UK-68798 (9) and risotilide (10) in ventricular muscle and of the effects of tedisamil on rat myocytes (2) and mammalian cardiac and glial cells (8). Some studies have been carried out to better determine the sites and molecular basis for the effects of the drugs in cardiac tissue. In preliminary experiments (using whole-cell patch clamp), UK-68798 (at 2 μM) decreased the amplitudes of outward tail currents in guinea pig ventricular myocytes (9). These results were suggested to be due to actions of the drug on I_K. It should be noted that in pharmacological and toxicological studies, at a wide range of doses, no obvious effects of UK-68798 on normal neuronal function have been observed (personal communication, Pfizer, Kent, England. Block of I_K was also suggested from preliminary voltage-clamp experiments on the actions of risotilide on cat ventricular myocytes (10). Recent studies using other sulfonamide agents have been described (11). Both *d*-solatol (100 μM) and a more potent structural analogue of solatol, E-4031 (IC_{50} near 0.4 μM), were found to block a rapidly activating component of the I_K in guinea pig ventricular myocytes. It would seem reasonable to assume that the greater potency for the blocking actions of E-4031, relative to solatol, could be attributable to increased lipid solubility of the former compound.

The best studied drug, of the three that have been applied here, is tedisamil. Whole-cell patch-clamp experiments have shown that tedisamil (at 1–20 μM) significantly hastened the decay phase of I_{TO} in rat ventricular myocytes (2). The actions on I_{TO} were independent of membrane potential. In addition, the drug was equally efficacious when applied from inside or outside the cells. Interestingly, I_{TO} in cardiac cells has been suggested to depend, at least partly, on calcium concentration (12, 13). More recently, tedisamil has been applied to guinea pig ventricular myocytes and mouse astrocytes (8). With the cardiac cells, tedisamil (at 3 or 10 μM) decreased I_K with a K_D of 2.5 μM (8). With the glial cells, tedisamil (at 10 μM) decreased both transient (I_A) and delayed (I_K) K^+ currents (8). These results were interpreted as voltage-independent channel block by tedisamil acting at a site accessible from the cytoplasm. A comparison of the data on tedisamil block of I_{TO} in cardiac cells and the present data on the block of $I_K(Ca)$ in hippocampal neurons shows a significant degree of commonality. This includes similar concentrations of drug applications that were effective in channel blockade, effects from the drug applied to the outside or the inside of the cell membrane, and drug actions that were independent of potential.

It is interesting to consider which macroscopic K^+ current may be associated with the single-channel properties measured in the present experiments. Such properties include channel kinetics dependent upon internal Ca^{2+} but not on patch potential and also no obvious inactivation over long periods of recording. The most likely candidate is I_{AHP}, which underlies the slow afterhyperpolarization observed in CA1 pyramidal neurons (14, 15). The I_{AHP} is dependent on entry of extracellular Ca^{2+} and, once activated, is not turned off with repolarization (13). In addition, the time constants for the decay of I_{AHP} are insensitive to voltage (14). Finally, I_{AHP} showed little sensitivity to applications of external TEA (up to 2 mM); in the present experiments, TEA (at 2 mM) did not alter channel kinetics with applications to inside-out or outside-out patches.

Acknowledgments

The authors wish to express their thanks to Dr. K. Baimbridge and Ms. S. Atmadja for the preparation and maintenance of the cultured cells used in this work and to Dr. M. J. A. Walker for assistance with some of the experiments.

References

1. McLarnon, J. Block by a putative antiarrhythmic agent of a calcium-dependent potassium channel in cultured hippocampal neurons. *Neurosci. Lett.* **112**:210–215 (1990).
2. Dukes, I. D., and M. Morad. Tedisamil inactivates transient outward K^+ current in rat ventricular myocytes. *Am. J. Physiol.* **257**:H1746–H1749 (1989).
3. Beatch, G. N., S. Abrahams, B. A. MacLeod, N. R. Yoshida, and M. J. A. Walker. Antiarrhythmic properties of tedisamil (KC 8857), a putative transient outward K^+ current blocker. *Br. J. Pharmacol* **102**:13–18 (1991).
4. Banker, G. A., and W. M. Cowan. Rat hippocampal neurons in dispersed cell culture. *Brain Res.* **126**:397–425 (1977).
5. Findlay, I., M. J. Dunne, and O. H. Petersen. High-conductance K^+ channel in pancreatic islet cells can be activated and inactivated by internal calcium. *J. Membr. Biol.* **83**:169–175 (1985).
6. Brown, P. D., D. D. F. Loo, and E. M. Wright. Ca^{2+}-activated K^+ channels in the apical membrane of *Necturus* choroid plexus. *J. Membr. Biol.* **105**:207–219 (1988).
7. Colatsky, T. J., and C. H. Follmer. K^+ channel blockers and activators in cardiac arrhythmias. *Cardiovasc. Drug Rev.* **7**:199–209 (1989).
8. Dukes, I. D., L. Cleemann, and M. Morad. Tedisamil blocks the transient and delayed rectifier K^+ currents in mammalian cardiac and glial cells. *J. Pharmacol. Exp. Ther.* **254**:560–569 (1990).
9. Gwilt, M., H. W. Dalrymple, R. A. Burges, K. J. Blackburn, J. E. Arrowsmith,

P. E. Cross, and A. J. Higgins. UK-68,798 is a novel, potent and selective class III antiarrhythmic drug. *J. Mol. Cell. Cardiol.* **21**:S11 (1989).
10. Follmer, C. H., M. T. Poczobutt, and T. J. Colatsky. Selective block of delayed rectification (I_K) in feline ventricular myocytes by WY-48,986, a novel class III antiarrhythmic agent. *J. Mol. Cell. Cardiol.* **21**:S185 (1989).
11. Sanguinetti, M. C., and N. K. Jurkiewicz. Two components of cardiac delayed rectifier K^+ current: differential sensitivity to block by class III antiarrhythmic agents. *J. Gen. Physiol.* **96**:195–215 (1990).
12. Siegelbaum, S. A., and R. W. Tsien. Calcium-activated transient outward current in calf cardiac Purkinje fibres. *J. Physiol. (Lond.)* **299**:485–506 (1980).
13. Coraboeuf, E., and E. Carmeliet. Existence of two transient outward currents in sheep cardiac Purkinje fibres. *Pflügers Arch.* **392**:352–359 (1982).
14. Lancaster, B., and P. R. Adams. Calcium-dependent current generating the afterhyperpolarization of hippocampal neurons. *J. Neurophysiol.* **55**:1268–1282 (1986).
15. Storm, J. Action potential repolarization and a fast afterhyperpolarization in rat hippocampal pyramidal neurons. *J. Physiol. (Lond.)* **385**:733–759 (1987).

Send reprint requests to: James McLarnon, Department of Pharmacology and Therapeutics, Faculty of Medicine, The University of British Columbia, Vancouver, BC, Canada.