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Block of Heart Potassium Channels by Clofilium and Its Tertiary Analogs: Relationship between Drug Structure and Type of Channel Blocked

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SUMMARY

The whole-cell arrangement of the patch clamp was used to study delayed rectifier and inward rectifier K channels in isolated guinea pig ventricular cells. Block of these channels by an externally applied quaternary nitrogen compound, clofilium, and two of its tertiary nitrogen structural analogs (LY97241 and LY97119) were investigated. Clofilium reduced delayed rectifier current but had little effect on inward rectifier currents in concentrations as high as 100 μM. The block of delayed rectifier did not reverse upon washout. In contrast, lower concentrations of the tertiary analogs blocked both delayed rectifier and inward rectifier K currents. Onset of block of delayed rectifier was fast and block was reversible. The onset of block of inward rectifier by the tertiary compounds was slower than for delayed rectifier current and more difficult to reverse. We conclude from this work that tertiary, but not quaternary, clofilium blocks inward as well as delayed rectifier channels in these cells. Block of inward rectifier current is presumably caused by access to a receptor for the molecule that is gained by the tertiary, but not the quaternary, forms of the drug.

Potassium channel currents are critical to the regulation of electrical activity in excitable cells because they regulate the action potential duration and speed of repolarization and determine cellular resting potentials. K channels are particularly important in the heart, where several types of K channels are now known to exist. This diverse group includes the following: 1) I_{IR} (1, 2), 2) I_{DR} (3, 4), 3) ATP-regulated (5–7), and 4) Na-activated (8) channels. In addition, I_{to} may be in part a calcium-activated K channel current (9).

Despite this diversity and importance to cellular function, molecular and biophysical studies of K channels in the heart lag behind those of Na and Ca channels, due, in part, to the absence of potent and specific pharmacological probes. Compounds that are presently used to block K channels in heart cells are applied in high concentrations (1–100 mM) and generally block more than one K channel type. For example, external application of the inorganic cations Ba^{2+} and Cs^{+} block both I_{DR} and I_{IR} (10–14). Internally applied Mg^{2+} blocks both I_{IR} (15) and the ATP-regulated channel (16).

Some organic compounds, such as the QA derivatives TEA and tetrabutylammonium also block more than one K channel type. Internally applied QA compounds block I_{DR}, I_{to} (17), and the ATP-regulated channel (7). Externally applied TEA appears to block both I_{DR} and I_{IR} according to data obtained from action potential and voltage clamp studies (18–20). Another example is the antiarrhythmic drug quinidine, which has been found to block both I_{DR} and I_{IR} (21–23).

In this study, we focused on two of the K channel currents in the heart, I_{DR} and I_{IR}. We tested the sensitivity of both currents to a series of structurally related compounds in order to determine the structure-activity relationship necessary to cause selective block of these currents. As a starting compound in the study we chose clofilium, a quaternary nitrogen compound because, when applied externally, it has been shown to block I_{DR} in isolated guinea pig ventricular cells (24). We found that slight structural changes in this compound caused differential block of these two K channel currents. Some of the results of this study have appeared in abstract form (25).

Materials and Methods

Nomenclature. Potassium channels in the heart have been referred to by a variety of names [reviewed by Noble (26)]. The background inwardly rectifying current sometimes referred to as I_{K1} (27) is simply

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ABBREVIATIONS: I_{IR}, inward rectifier current; I_{DR}, delayed rectifier current; HEPES, *N*-2-hydroxyethylpiperazine-*N*-2-ethane sulfonic acid; TTX, tetrodotoxin; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N*,-tetraacetic acid; TCLOF, LY97119, the tertiary analog of clofilium; LY, LY97241, the *p*-nitro tertiary analog of clofilium; QA, quaternary ammonium; I_K, nerve delayed rectifier current; TEA, tetraethylammonium; I_{to}, transient outward current; V_K, K-equilibrium potential.

called the inward rectifier K channel current (I_{IR}) in this paper. The delayed potassium current, which has been called I_X in earlier work (3), is referred to as the delayed rectifier current I_{DR}).

We abbreviate the drugs used in this investigation as follows. Clofilium is referred to by its complete name. The tertiary analog (LY97119) is called TCLOF, and the *p*-nitro tertiary analog (LY97241) is called LY. The structures of these compounds are presented in Fig. 1.

Cell isolation and recording procedures. Single myocytes were isolated from either ventricle of adult male or female guinea pigs (Charles River, Charles River, MA) weighing approximately 200–350 g. The isolation procedure is that described by Mitra and Morad (28) except that we use 300–350 units/ml of collagenase type II enzyme (Worthington Biomedical Corporation, Freehold, NJ) and 0.3 mg/ml protease (Sigma type XIV; St. Louis, MO).

Recording methods were as described by Hamill *et al.* (29) for the whole cell configuration. Pipettes were made from Gold Seal Accu-fill 90 Micropets (Clay Adams, Inc., Parsippany, NJ). The resistance of the pipette was typically 1–3 MΩ when filled with K-pipette solution (see below). Series resistance compensation was used in all experiments and was adjusted to give the fastest possible capacity transients without producing ringing. Data were sampled at a range of frequencies (60 Hz to 3.3 kHz) depending on experimental protocols, filtered (30 Hz to 5 kHz) with an 8-pole Bessel filter (Frequency Devices, Haverhill, MA), digitized, and stored on a PDP-1123 computer (Digital Equipment Corp., Maynard, MA).

Recordings were made at room temperature (20–24°) from a Plexiglass chamber mounted on the stage of an Olympus IMT inverted microscope. Solutions in the chamber were changed with voltage-controlled valves (General Valve Corp., Fairfield, NJ) connected to syringe reservoirs. Solution was removed with suction. The chamber volume was 1 ml and approximately 15 ml of solution was washed through (in 1–2 min) for a solution change. In some experiments cells were perfused at a rate of 1.0 ml/min whereas in others the solution remained static between solution changes. Results were the same in both cases.

Solutions and drugs. Isolated cells were initially placed in a standard Tyrode's solution consisting of (mM): NaCl (132), KCl (4.8), $MgCl_2$ (3), $CaCl_2$ (1), glucose (5), HEPES (10), pH 7.4. After establishment of whole cell voltage clamp the solution was changed to Na-free solution (mM): Tris·HCl (140), Tris base (20), KCl (4.8), $MgCl_2$ (3), $CaCl_2$ (0.1), $CdCl_2$ (0.2), and dextrose (5), pH 7.3. Currents through Na channels were eliminated by addition of 10-50 μM TTX and by replacement of external Na with Tris. Ca currents were eliminated by the addition of 200 μM $CdCl_2$.

The K-pipette solution consisted of (mM): KCl (50), K glutamate (60), $MgCl_2$ (2), $CaCl_2$ (1), HEPES (10), EGTA (11), and K_2ATP (3) and was buffered to pH 7.3 with KOH (final K concentration, 140 mM). The free Ca^{2+} and Mg^{2+} concentrations of this solutions were 1×10^{-8} and 1.5×10^{-4} M, respectively. Na-free internal solution eliminated contributions from the Na-activated K channel (8) and the Na/K ATPase (30). The ATP-regulated K channel was suppressed by the presence of 3 mM ATP (7).

Clofilium, LY97119, and LY97241 were gifts of Lilly Research Laboratories (Indianapolis, IN). TTX was purchased from Behring Diagnostics (La Jolla, CA).

Voltage protocols. For most experiments the holding potential was set to the zero-current level. After correction for liquid junction potential (31) the mean zero-current potential of −81.6 ± 0.5 mV (32 experiments) was close to the predicted Nernst potential (−85 mV) for a K-selective electrode (V_K) after including a −3 mV offset for possible contributions of fixed internal cations (32). Some experiments were carried out at more depolarized holding potentials (−30 mV). We found no difference in drug action or in membrane currents measured in either set of experiments. Thus the transient outward current (I_{to}), which is fully inactivated at a holding potential of −30 mV, but not −80 mV, (33) does not contribute to the measured currents under the conditions of our experiments.

I_{IR} was measured at the end of brief (40 msec) voltage steps applied from the holding potential to eliminate overlap from I_{DR} which activates with a delay of about 200 msec (34, 35) and time constants on the order of seconds in isolated guinea pig cells (35, 36). Currents measured with this protocol consist of I_{IR} and leak. In some experiments, leak currents were measured directly in the presence of $BaCl_2$ (5–10 mM), which completely blocks I_{IR} (13, 14). In other experiments, we estimated leak currents by extrapolation of a linear curve fit to currents positive to 0 mV. Fig. 2 shows the excellent agreement between both methods. Experiments were only acceptable in cells in which leak currents were unchanged. Estimation of drug block was carried out after leak currents were subtracted from total current measurements.

I_{DR} was measured as the time-dependent current at the end of long (usually 2 sec) voltage pulses. Time-independent currents (leak plus I_{IR}) were measured at the beginning of each pulse and subtracted (see also Ref. 27).

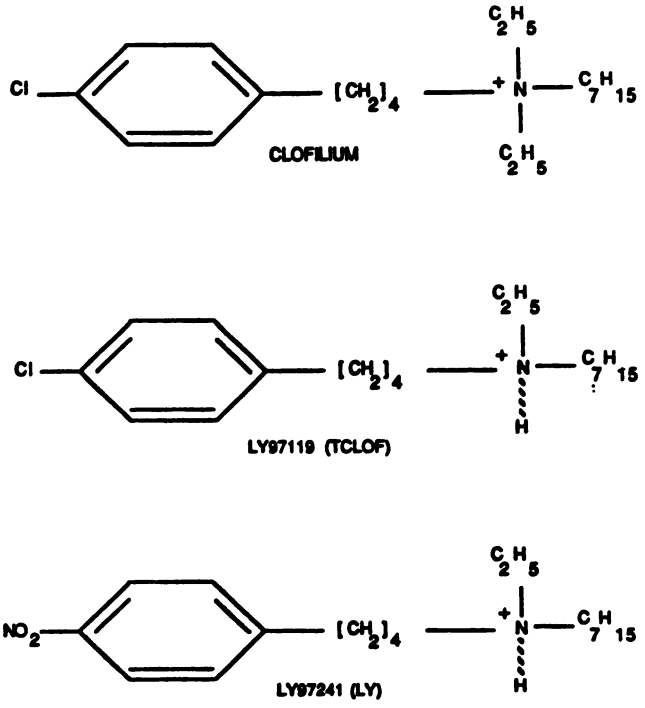

Fig. 1. Structures of clofilium (*upper*), TCLOF (*middle*), and LY (*lower*).

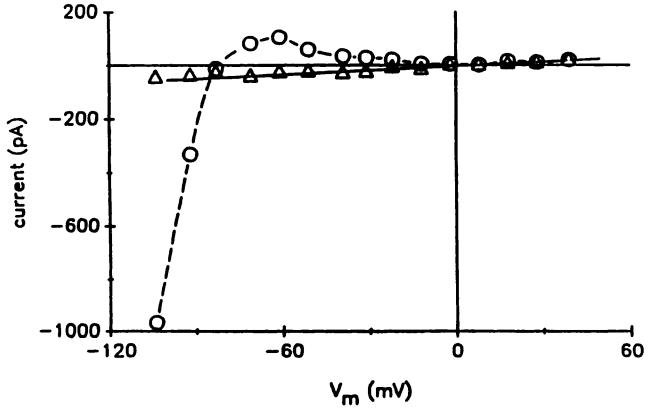

Fig. 2. Measurement and estimation of leak currents using two methods in a single preparation. Control currents measured at the end of 40-msec voltage pulses plotted against membrane potential (O). These currents are composed of I_{IR} and leak. Leak currents were measured directly 2 min after adding 5 mM $BaCl_2$ (Δ) to block I_{IR}. Leak current was also estimated by a linear regression fit to the control data positive to 0 mV (*solid line*). The two methods agree well. Holding potential was −83 mV. Cell no. 10272.

Current-voltage relations that are shown for I_{DR} are actually isochronal curves that show I_{DR} activated at fixed times (indicated in the respective figures) but over a range of voltages.

Estimation of drug block. Control data were taken 5 min after changing to Na-free extracellular solution. The bath solution was then changed to drug-containing Na-free solution and unless otherwise indicated currents were measured 5 min later. Where indicated, the bath solution was changed back to drug-free solution to test for reversibility of drug block.

In some cells, we found that I_{DR} decreased over the time course of our experiments in a manner that paralleled rundown of Ca channel currents in similar preparations (37, 38). In control experiments rundown followed for up to 30 min was best fit with a single exponential time course (average time constant was 134 ± 30 sec, range 55 to 245 sec, eight experiments). Therefore, during the first 5 min the current declined to 70 to 99% of its steady-state value. Data collection began after this initial fast phase of rundown. When necessary, we used the time constant calculated from 5 min of predrug data to extrapolate current level at the time block was estimated. In many instances this extrapolation was not necessary because the time course of block was sufficiently fast compared with rundown rate.

As has been reported by others (23) we found that inward current through I_{IR} channels decreased with time during our experiments. In contrast, outward current through these channels was very stable over the duration of our experiments. Because of this stability we chose to measure block of I_{IR} at voltages positive to V_K in most of our experiments. However, measurement of block of I_{IR} at voltages negative to V_K provided results similar to those obtained at more positive voltages.

Data analysis. All averaged data that are included in text of figures are mean ± standard error. A paired t test was used to compare control and drug groups ($p < 0.05$ significance level). Dose-response curves were fit using a nonlinear least squares fitting program (39) to a modified Michaelis-Menten equation:

$$\% \text{ block} = 1/(1 + (K_d/[D])) \quad (1)$$

where [D] is the drug concentration and K_d is the drug concentration for half-maximal effect.

Results

Clofilium blocks I_{DR} but not I_{IR}. The reduction of I_{DR} by clofilium is illustrated in Fig. 3. The *inset* shows I_{DR} in the absence and presence of 50 μM clofilium. Also shown is I_{DR} measured at the end of a series of 2-sec voltage pulses and plotted against pulse voltage. In this experiment, I_{DR} measured at +40 mV was reduced 60% by the drug, a reduction similar to the mean of 50.8 ± 5.0% (five cells). We also tested for but found no significant difference between drug block at different voltages. We found clofilium concentrations greater than 100 μM were toxic to our cells, limiting the useful concentration range of this drug. Nevertheless, we did measure a slight increase in block at 100 μM (56.7 ± 4.6% of I_{DR} in seven cells) and found no significant reduction of current at a lower drug concentration (10 μM). We found the effects of clofilium on I_{DR} did not recover for washout periods of up to 20 min.

Although 50 μM clofilium blocks approximately 50% of I_{DR}, it is ineffective in blocking I_{IR}. Fig. 4 illustrates this point. The *inset* consists of current records in response to voltage steps that are positive and negative to V_K, showing I_{IR} in the outward and inward directions. The records, shown before (*left*) and after (*right*) exposure to 50 μM clofilium, indicate that the drug has little effect on current through this channel at these voltages. This insensitivity is confirmed over a range of voltages in the current voltage relation plotted in the figure. The small reduction in current at voltages negative to the reversal potential is due to the instability of current in the inward direction through the I_{IR} channels (see Materials and Methods).

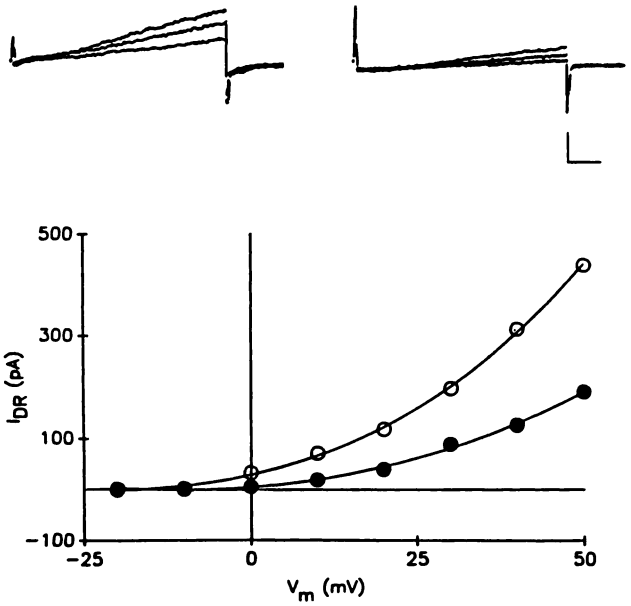

Fig. 3. Clofilium blocks I_{DR}. *Inset*, I_{DR} in response to 2-sec voltage steps to +30, +40, and +50 mV (*bottom* to *top*) in the absence (*left*) and presence (*right*) of 50 μM clofilium. Calibrations are 250 pA and 300 msec. Holding potential was −81 mV. Leak subtraction as described in Materials and Methods. *Curve*, currents measured at the end of 2-sec pulse versus pulse potential. ○, Control; ●, 50 μM clofilium. Cell no. 4301.

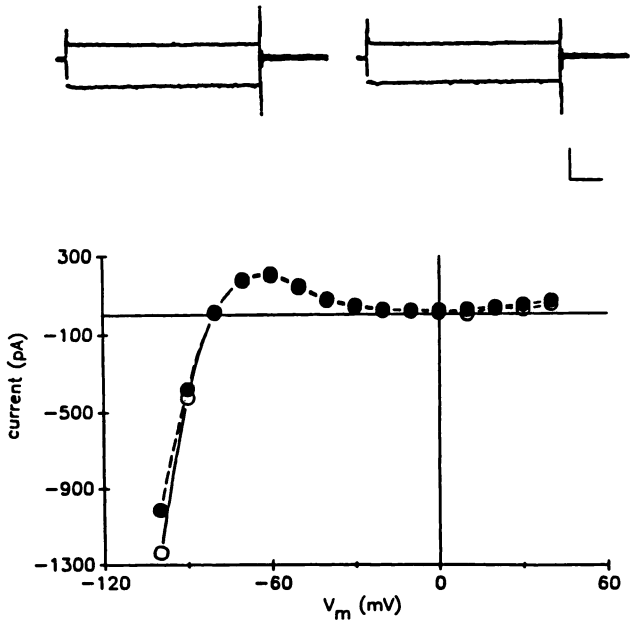

Fig. 4. Clofilium does not block I_{IR}. *Inset*, currents in response to 40-msec pulses to −60 mV (*upper* traces) and −90 mV (*lower* traces) in the absence (*left*) and presence (*right*) of 50 μM clofilium. Holding potential was −82 mV. Calibrations are 500 pA and 7 msec. *Curve*, currents measured at the end of 40-msec pulses plotted against pulse voltage. ○, Control. ●, 50 μM clofilium. Cell no. 1252.

In summary, we found I_{IR} was insensitive to clofilium over a concentration range of 10 to 100 μM. Block of this current was not observed even if the exposure time of the highest concentration (100 μM) was extended to 20 min. We thus conclude from this work that clofilium blocks I_{DR} as reported previously

by Snyders and Katzung (24), and that I_{IR} is little affected over the concentration range studied.

Block of both I_{DR} and I_{IR} by the tertiary derivatives of clofilium. We next tested the sensitivity of currents through these two channels to the *p*-nitro, tertiary nitrogen structural analog of clofilium, LY (Fig. 1). We first studied the effects of this compound of I_{DR}. Fig. 5 illustrates this point by presenting the results of an experiment in which the effects of 50 μM LY were measured on I_{DR} (compare with Fig. 3 for similar clofilium concentration). Here I_{DR} was reduced nearly 10-fold, suggesting that this derivative is much more potent than the quaternary compound. The potency of LY was confirmed in four similar experiments in which the mean reduction of I_{DR} in 50 μM LY was 89.6 ± 4.4%.

We next investigated the sensitivity of I_{IR} to LY and found it strikingly different from that to clofilium. LY reduces I_{IR} at roughly the same potency as it inhibits I_{DR}. An example of its actions on I_{IR} is shown in Fig. 6. Illustrated in Fig. 6 *inset* are currents recorded in the absence and presence of LY (50 μM) at voltages negative and positive to V_K. At these voltages, and over the range of voltages tested, most of I_{IR} (>85%) was blocked. The mean block at −50 mV for four such experiments was 86.7 ± 6.7% compared with 89.6 ± 4.4% for I_{DR}.

As discussed previously, LY is the *p*-nitro, tertiary nitrogen analog of clofilium. We were able to test the importance of the *p*-nitro substitution by studying the effects of the *p*-chloro tertiary derivative of clofilium, TCLOF (see Fig. 1 for structure), on I_{DR} and I_{IR}. In the steady state, we found that this compound resembled LY in its inhibition of I_{IR} and I_{DR}. TCLOF (50 μM) reduces both I_{DR} (87.0 ± 2.4%, eight experiments) and I_{IR} (71.0 ± 5.3%, five experiments).

Fig. 7 shows that the potency to block either current does not appear to be affected by the *p*-nitro substitution. The figure shows the fraction of current blocked plotted against drug

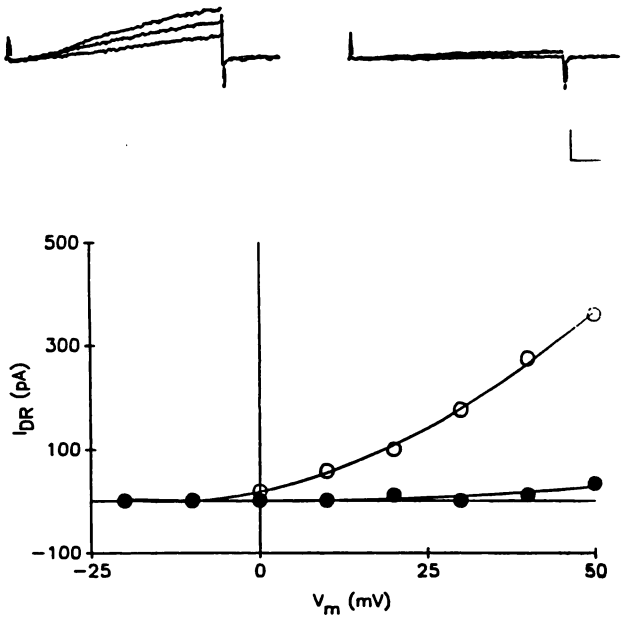

Fig. 5. LY blocks I_{DR}. *Curve*, I_{DR} measured at the end of 2-sec voltage pulses plotted against pulse potential. ○, Control. ●, 50 μM LY. *Inset*, I_{DR} in response to 2-sec voltage pulses to +30, +40, and +50 mV (*bottom* to *top*) in the absence (*left*) and presence (*right*) of 50 μM LY. Holding potential was −81 mV. Leak subtraction as described in Materials and Methods. Calibrations are 225 pA and 300 msec. Cell no. 4243.

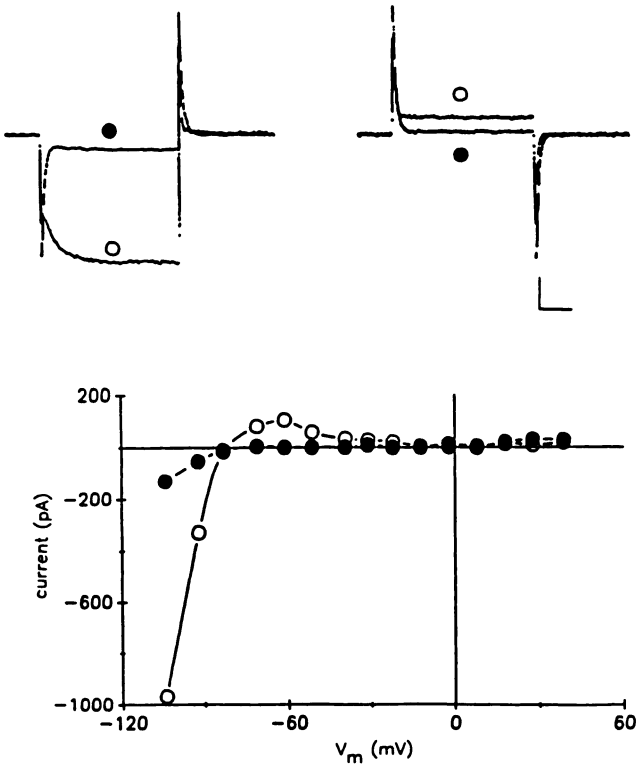

Fig. 6. LY blocks I_{IR}. *Curve*, currents measured at the end of 40-msec pulses plotted versus pulse voltage in control (○) and LY (50 μM)-containing solutions (●). *Inset*, currents in response to 40-msec pulses to −100 mV (*left*) and −62 mV (*right*). Symbols from curve indicate control and LY traces. Holding potential was −83 mV. Calibrations are 250 pA and 9 msec. Cell no. 1027.

concentration for both I_{DR} and I_{IR} channel types. The drugs are virtually equipotent in blocking each of these channels. The smooth curves in the figure represent the best fit of a single binding site (Eq. 1) to the LY data, but the agreement with the TCLOF data is clear. LY was slightly more potent in blocking I_{DR} (K_d = 6.4 μM) than I_{IR} (K_d = 12.4 μM).

Time course of block onset and recovery: differences between the compounds. Fig. 8 presents the time course of onset of and recovery from block of I_{DR} and I_{IR} by the three compounds. Differences between the compounds emerge when these data are compared. In these experiments, currents were measured during voltage pulses applied once every 15 sec. Currents were measured under control conditions and as the external solution was changed to drug-containing and then drug-free solutions. Current was normalized to control magnitude and plotted against time.

As discussed previously, Fig. 8A shows that the parent quaternary compound clofilium does not affect I_{IR} but does reduce I_{DR}. The experiment summarized in this figure illustrates the effects of the highest clofilium concentration (100 μM) studied. The block of delayed rectifier that develops is very difficult to reverse, even for washout periods up to 20 min.

The tertiary forms of the drug inhibit both currents. For 50 μM LY (Fig. 8B), the *p*-nitro-substituted derivative, the reduction of I_{DR} occurred as soon as we measured currents after the solution was changed and steady-state effects (about 85% block) were reached with a half-time of 15 sec in this experiment (mean of 27 ± 6 sec, five cells). Recovery from block occurred with roughly the same time course (half-time, 20 sec

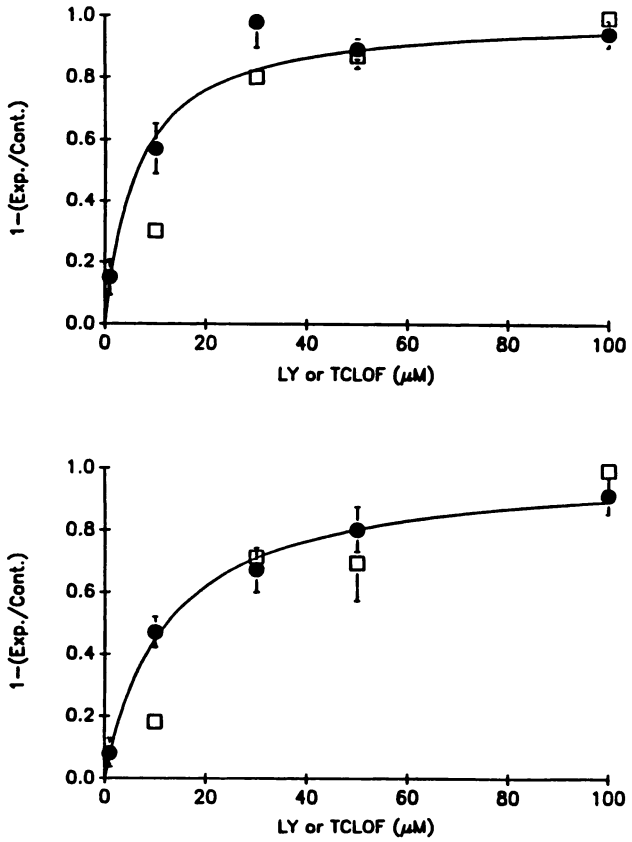

Fig. 7. Dose-response relationships for block I_{DR} and I_{IR} by tertiary clofilium analogs, LY and TCLOF. Fraction of current blocked is plotted against drug concentration for delayed rectifier (*upper panel*) and inward rectifier (*lower panel*) currents. □, TCLOF data; ●, LY data. The *smooth curve* in each panel is the best fit of the LY data to Eq. 1. TCLOF data without error bars represents two determinations. The K_d values were 6.4 μM for I_{DR} and 12.4 μM for I_{IR}.

in this experiment) when the external solution was returned to drug-free conditions. The time course of the changes in I_{IR} induced by LY were slower than comparable changes in I_{DR}. For example, the half-time to block was 91 ± 33 sec. Recovery of I_{IR} from LY block was very slow and often incomplete (for washout periods up to 10 min).

TCLOF (50 μM) (Fig. 8C) also blocks both currents and its onset time course resembles that of the same LY concentration. In this case, half-time was 40 ± 9 sec (10 cells) for I_{DR} and 103 ± 15 sec (seven cells) for I_{IR}. It took at least 7 min to reverse TCLOF block of I_{DR} and complete recovery was never attained. Furthermore, block of I_{IR} by TCLOF was very stable. We measured little or no recovery of I_{IR} block by TCLOF in all experiments, even after prolonged (10 min) periods of washout.

Voltage- and time-independent block by clofilium and its analogs. Fig. 9 is an example of an experiment in which we tested for time-dependent block of I_{DR} by LY. The *insets* show the effects of LY (10 μM) on currents recorded in response to 4-sec pulses to +50 mV. The records on the *left* were obtained in the absence and presence of LY. On the *right*, the current trace recorded in the presence of LY was multiplied by a scale factor (3.3) and superimposed on the control trace. The agreement between the scaled and control traces indicates that a constant fraction of current was blocked during the time course of the pulse (4 sec). This lack of time dependence was evident at all voltages studied. This is illustrated in the *lower* part of

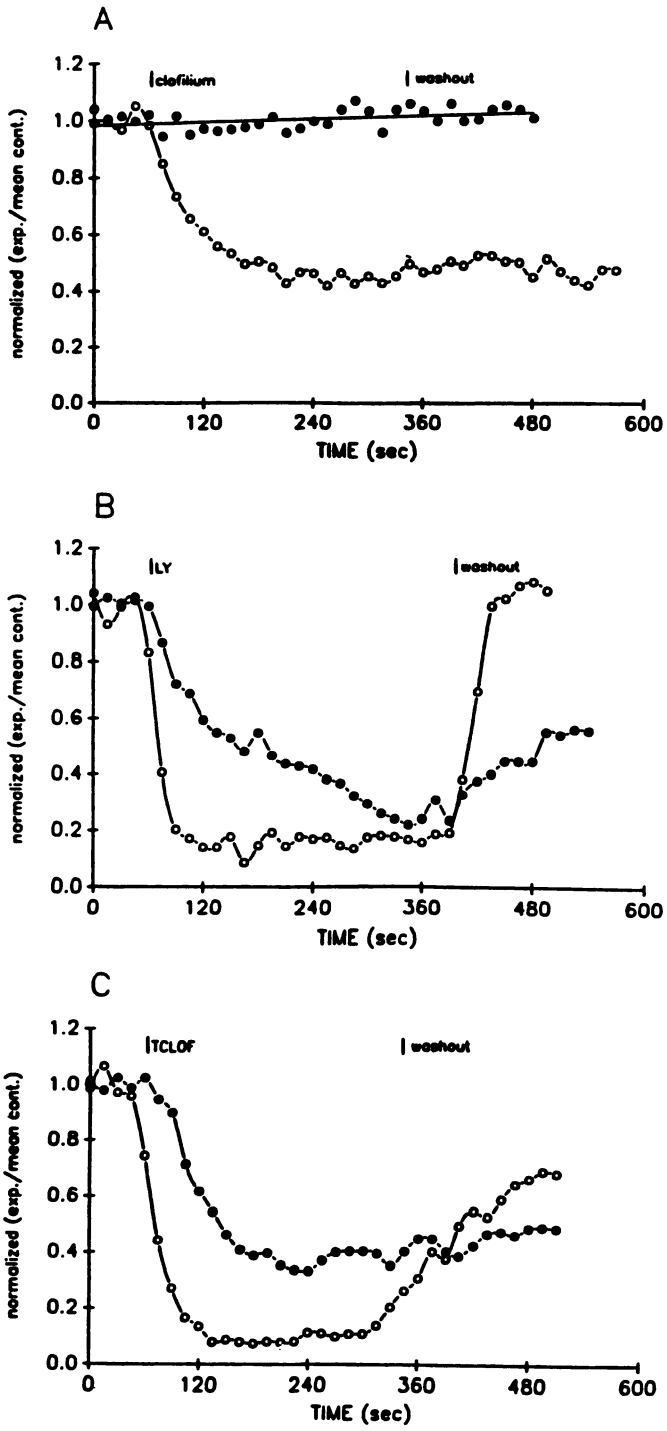

Fig. 8. Time course for onset of and recovery from I_{DR} and I_{IR} block by clofilium and its tertiary analogs. I_{IR} measured at the end of 40-msec pulses to −50 mV, and I_{DR}, measured at the end of 2-sec pulses to +50 mV were normalized to predrug values and plotted against time after changing to drug-containing solutions. Currents were measured every 15 sec. ○, Normalized I_{DR}; ●, normalized I_{IR}. Results are shown for (A) clofilium (100 μM), (B) LY (50 μM), and (C) TCLOF (50 μM). In each case, holding potentials were near −80 mV. *Straight line* in A is linear regression fit. Cell numbers are (A) 7303; (B) 4232; (C) 8201.

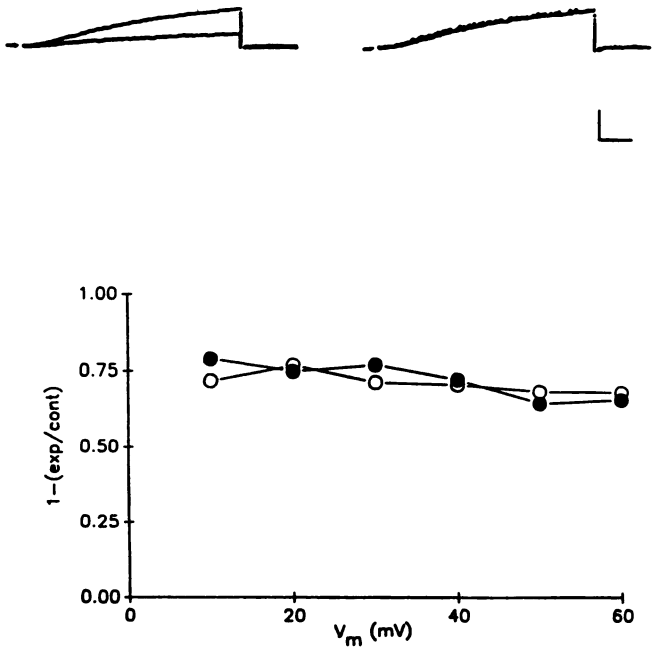

Fig. 9. Lack of time- or voltage-dependent block of I_{DR} by LY. *Insets*, the *left* records show I_{DR} in response to 4-sec voltage pulses to +50 mV in the absence (*upper trace*) and presence of 10 μM LY (*lower trace*); on the *right*, drug-containing trace was scaled by a factor of 3.3 and superimposed on the control trace. Calibrations are 550 pA and 650 msec. Leak subtraction as described in Materials and Methods. The first and last 6 msec of each current record has been blanked. *Curve*, fraction of I_{DR} blocked by LY (50 μM) measured at the end of 2- (○) and 4-sec (●) pulses plotted against pulse voltage. Same experiment as in *insets*. Holding potential was near −80 mV. Cell no. 8251.

Fig. 9, in which the amount of block developed at 2 and 4 sec was measured during a series of voltage pulses. The fraction of current blocked is the same at 2 sec as it is at the end of each voltage pulse.

Fig. 9 also illustrates the absence of voltage-dependence for block of I_{DR}. The fraction of current blocked is approximately 0.75 over the voltage range studied. Using a similar protocol, we found no evidence for either a voltage- or time-dependent block of I_{DR} by clofilium and TCLOF (data not shown). Tests for voltage- and time-dependent block of I_{IR} by each compound were also negative.

I_{IR} and I_{DR} are insensitive to intracellular application of drugs. We carried out a set of experiments in which we included high concentrations of clofilium (100 μM) or LY (50 μM) in our pipette solutions to test the sensitivity of both I_{DR} and I_{IR} to internally applied compounds. In these experiments, we began collecting data immediately after establishment of whole-cell clamp and monitored changes in each current for periods up to 30 min. Kameyama *et al.* (40) have used a simple compartmental model to show that substances with molecular weights similar to LY and clofilium such as GTP and cAMP (molecular weights, 400–500) reach 90% of the pipette concentration within 5 min in the intracellular medium under experimental conditions similar to ours. Nevertheless, we failed to measured inhibition of either current even after prolonged periods of dialysis in any experiment (five experiments for clofilium, three experiments for LY).

Discussion

Relationship between structure and blocking activity. The principal new result we present in this study is that block of K channels in heart cells can be modified by slight structural changes in a parent quaternary ammonium compound, clofilium. We found that the type of channel blocked, the relative potency, and the onset of and recovery from block were markedly changed by using tertiary and *p*-nitro-phenyl-substituted analogs of clofilium. This is the first report of such modification in K channel-blocking activity in heart cells that can be demonstrated by a slight structural modification of a blocking compound.

The clearest difference in drug activity between the quaternary (clofilium) and the tertiary (LY and TCLOF) analogs was the insensitivity of I_{IR} channels to the quaternary form of the drug. Clofilium failed to substantially inhibit current through this channel despite prolonged exposure (up to 20 min) and high drug concentrations (100 μM). On the other hand, lower concentrations of both LY and TCLOF rapidly blocked both I_{IR} and I_{DR}.

In addition to this change in the type of channel blocked, differences emerged in the time course of both the onset of and recovery from block. We found that I_{DR} failed to recover from clofilium-induced block, but the inhibition was reversible when tertiary analogs were used. Interestingly, inhibition of I_{IR} was slower in onset and recovery than the corresponding block of I_{DR} by either of the tertiary compounds. In general, the effects of TCLOF were more difficult to reverse than those of LY I_{IR} block by TCLOF was irreversible over 10-min washout periods.

Comparison to quaternary ammonium block of nerve delayed rectifier K channels. Armstrong and co-workers first used QA compounds to probe the architecture and gating properties of delayed rectifier K channels (I_K) in nerve (for reviews see Refs. 41 and 42). From these studies, and those which followed by other investigators (43, 44), several characteristics of intracellular QA block have emerged. QA compounds gain access to a receptor from the internal side of the cell membrane only after I_K channels open. This block produces a voltage- and time-dependent decrease in I_K during depolarizing voltage pulses that is characterized by concentration-dependent changes in blocking rate. Unblock occurs at negative potentials and is speeded if K^+ ions enter the pore from the extracellular side of the membrane (inward I_K). The results of these experiments were used to support the hypothesis that these compounds enter the K channel pore from inside the cell and physically block ion movement. Pore structure was then studied by using compounds of varying dimensions (41, 43, 44).

Our results suggest that block of I_{IR} and I_{DR} by clofilium and its analogs differs from intracellularly mediated QA block of nerve K channels. First, we found that LY and clofilium were ineffective when applied intracellularly. Second, we tested for but did not find evidence for voltage- and time-dependent block by these compounds. It can be argued that the fast kinetics of voltage-dependent QA block of delayed rectifier observed in nerve (41, 43, 44) would be obscured by the very slow kinetics of the cardiac delayed rectifier channel (3, 4). However, this is not the case for I_{IR}. Here, we found no change in block even after negative conditioning pulses were used to generate large inward movement of K^+ ions. If I_{IR} block resembled internal QA block of nerve I_K, this protocol should have modified the amount of I_{IR} blocked.

Because the drugs we studied do not appear to enter and plug the two K channels we investigated, it seems unlikely that these compounds can be used to provide structural information

about heart K channels in a manner that parallels previous work in nerve (41–44).

The block of I_{IR} and I_{DR} by clofilium and its analogs may more closely resemble the externally mediated QA block of K channels that is also described in nerve cells. External application of QA compounds produces time- and voltage-independent block of nerve K channels, and sensitivity of an external receptor varies considerably with different cell types (42).

Finally, the usefulness of these compounds as specific K channel blockers is limited because we find that other ion channels, such as Ca channels, are blocked in addition to K channels. This is not too surprising because even TEA has been shown to block nerve sodium channels under certain conditions (45).

Nevertheless, the differential inhibition of I_{IR} and I_{DR} by analogs of clofilium is a first step in characterizing the structure-activity relationships of compounds that can preferentially block these type of K channels. It will be interesting to modify portions of the molecule other than the quaternary end. Some clues may come from the time course of recovery of each K current from block by the tertiary clofilium analogs. That I_{IR} recovery from block is slower than recovery for I_{DR} suggests different off-rates for binding to the drug receptors responsible for block of the two channels. In addition, the removal of the *p*-nitro-phenyl substitution of the tertiary analog further slows recovery from block of I_{IR} and suggests that modification of this portion of the drug molecule might result in a more selective probe for the inward rectifier channel.

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