A novel enhancing effect of clofilium on transient outward-type cloned cardiac K^+ channel currents

¹Tsutomu Kobayashi, Gabor Mikala & Atsuko Yatani

Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0575, U.S.A.

1 The antiarrhythmic drug, clofilium, has been shown to block several types of K^+ channel currents. To investigate the effects of clofilium on the transient outward K^+ current (I_{to}), a cloned I_{to} -type cardiac K^+ channel (RHK1) was expressed in *Xenopus* oocytes and the drug effects were examined on whole cell currents.

2 Extracellular application of clofilium slightly inhibited the current at +60 mV from a holding potential of –80 mV. However, it unexpectedly enhanced the current from a holding potential of –60 mV in a dose-dependent manner ($219 \pm 39\%$ of control at 100 μ M).

3 This enhancement is probably due to an increase in the ratio of channels in the resting state during steady depolarization, since clofilium shifted the inactivation curve in the depolarizing direction.

4 LY97119, a tertiary ammonium analogue of clofilium, did not exhibit this enhancing effect but only inhibited the current.

5 Clofilium may be useful for the study of channel inactivation because this type of phenomenon has not been reported for any other drug.

Keywords: K^+ channel; transient outward; inactivation; antiarrhythmic drug; clofilium

Introduction

Clofilium (Figure 1) is one of the class III antiarrhythmic agents, which block K^+ channels in cardiac myocytes, prolong the action potential duration and exhibit antiarrhythmic effects. This compound blocks the delayed rectifier (I_K) (Arena & Kass, 1988; Reeve & Peers, 1992), the ATP-sensitive K^+ channels (Sakuta *et al.*, 1993) and I_{K1} (Folander *et al.*, 1990). The drug has also been reported to block the transient outward current (I_{to}) in isolated ventricular myocytes, but a long exposure time was required for its action (Castle, 1991).

I_{to} is assumed to be very important in the function of cardiac cells, since it determines the membrane potential of the plateau phase during the action potential that modulates other following voltage-dependent ion channels (Ten Eick *et al.*, 1992). However, the presence of multiple overlapping ion channels in native cardiac cells complicates the study of drug-channel interactions (Colansky *et al.*, 1990).

In an attempt to overcome some of these difficulties, we have examined the inhibitory effects of clofilium on a cloned I_{to} -type cardiac K^+ channel (a *Shaker* type, RHK1) (Tseng-Crank *et al.*, 1990) expressed in *Xenopus* oocytes. During the course of this study, we found that clofilium unexpectedly and dramatically enhanced the current when the resting membrane potential was slightly depolarized and the channels were partially inactivated. This phenomenon is interesting because this type of effect has not been reported for clofilium or any other drugs. This paper describes the enhancing effect on a cloned I_{to} current RHK1 by clofilium that has been known as a K^+ channel blocker.

Methods

The cDNA encoding RHK1 was previously isolated from a rat heart cDNA library (Tseng-Crank *et al.*, 1990). Runoff transcripts of the cRNA were prepared and microinjected

into *Xenopus* oocytes in portions of 50 nl per oocyte ($50\text{--}100\text{ ng }\mu\text{l}^{-1}$). After injection, the oocytes were maintained at 15–20°C in modified Barth's medium (in mM: NaCl 96, KCl 2, CaCl_2 1.8, MgCl_2 1, sodium pyruvate 2.5, theophylline 0.5, pH 7.3 with Tris base) in the presence of penicillin (100 units ml^{-1}), streptomycin ($100\text{ }\mu\text{g ml}^{-1}$) and gentamicin ($50\text{ }\mu\text{g ml}^{-1}$). These cells were used for experiments within 1 to 3 days after injection.

A two-microelectrode voltage clamp method was used with 3 M KCl-filled electrodes (1 to 2 M Ω) connected to a voltage clamp amplifier (Axoclamp 2A, Axon Instruments Inc, Burlingame, CA, U.S.A.) to measure the whole cell K^+ current. The pCLAMP software (Axon Instruments) was used for data acquisition and analysis. Currents were digitized at 5 kHz after being filtered at 1 kHz. To remove whole cell leak and capacitive currents, pulse protocols contained four hyperpolarizing pulses of one-quarter the test pulse amplitude before a test pulse, unless otherwise indicated. The current responses to all five of these pulses were summed by the pCLAMP system (the P/4 procedure). Recovery from inactivation of RHK1 required longer than 10 s, and voltage-clamp pulses were applied every 15 or 20 s.

A low Cl external solution was used to minimize Ca-activated Cl currents (in mM: N-methyl-D-glucamine 120, methanesulphonic acid 120, KOH 2.5, MgCl_2 2 and HEPES

Figure 1 Chemical structures of clofilium and LY97119.

¹ Author for correspondence at present address: Pharmacological Research Laboratory, Tanabe Seiyaku Co. Ltd., 2-2-50 Kawagishi, Toda, Saitama 335, Japan.

10, pH 7.4 with Tris base). The experimental chamber (0.3 ml in volume) was perfused continuously with gravity flow at a rate of 2 ml min⁻¹. The solution in the chamber was replaced within 30 s. All experiments were performed at room temperature (20–22°C).

Clofilium tosylate obtained from Research Biochemicals Inc. (Natick, Mass, U.S.A.) and LY97119 (Figure 1) donated by E. Lilly (Indianapolis, U.S.A.) were dissolved in ethanol at 10 and 100 µM and diluted to a final concentration with the external solution. Ethanol did not show any effect on currents even at the highest concentration (0.3%).

The experimental values were expressed as means ± s.e. mean. Statistical significance was tested by Student's paired, two-tailed *t* test, unless otherwise indicated.

Results

Effects of clofilium on *I*_{to} from a holding potential of –80 mV

The oocytes injected with the cRNA encoding RHK1 exhibited K⁺ currents that showed rapid activation and inactivation in response to depolarizing voltage clamp pulses. Because these currents are inactivated easily at a positive holding potential, we first tested the effects of clofilium at a holding potential of –80 mV. Figure 2 shows the effects of clofilium (10, 100 µM) on RHK1 currents elicited by successive depolarizing pulses to +40 mV from a holding potential of –80 mV. Application of clofilium did not influence the peak amplitude nor the activation rate. However, it accelerated the inactivation of currents in a concentration-dependent manner. Under the control conditions, the time course of inactivation could be fitted to a single exponential function with time constants of 33.5 ± 1.8 and 34.3 ± 1.9 ms (*n* = 5) at +20 and +60 mV, respectively. Four minutes after perfusion with 100 µM clofilium, the time course was also well-fitted to a single exponential function. However, the time constants decreased significantly to 27.4 ± 0.8 ms (*n* = 5, *P* < 0.02) and 25.3 ± 1.0 ms (*n* = 5, *P* < 0.01) at +20 and +60 mV, respectively.

Figure 2 Effects of clofilium (10, 100 µM) on the current of cloned transient outward-type K⁺ channel (RHK1) in oocytes injected with RHK1 cRNA. Currents were elicited by 250 ms depolarizing pulses (+40 mV) from a holding potential of –80 mV at intervals of 20 s. (a) Original current tracings before (C) and during superfusion with 10 and 100 µM clofilium (10,100) and after washout (W). (b) Time course of the effects on the *I*_{to} amplitude (●) and the rate of current inactivation (○).

Effects of clofilium on *I*_{to} from a holding potential of –60 mV

The capability of many compounds to block voltage-dependent ion channels is strongly affected by the holding potential in voltage-clamp experiments. For example, dihydropyridines block Ca²⁺ channels more strongly in depolarized cells (Bean, 1984). Therefore, oocytes were maintained at a holding potential of –60 mV, and the effects of clofilium were tested by successive depolarizing pulses at an interval of 20 s. Figure 3 shows the time courses of the onset of and the recovery from the effects of clofilium. Clofilium dramatically enhanced the currents in a dose-dependent manner. In view of the speed of solution replacement in the experimental chamber, the current amplitude increased and reached a new steady state by the time we measured currents after exposure to clofilium. Recovery from the elevated amplitude occurred with almost the same speed as the onset of enhancement. At the holding potential of –60 mV, the acceleration of current inactivation by clofilium was observed as was seen at a holding potential of –80 mV. The time courses of the onset of and recovery from this effect were slower than those of the enhancement effect on the current amplitude.

The enhancement of current amplitude by clofilium was observed at a wide range of depolarizing pulse potentials from –20 to 60 mV, but was not at the pulse potential of –40 mV (Figure 4). The peak amplitudes were 268 ± 54 and 1478 ± 276 nA (*n* = 5) at depolarizing pulse potentials of –20 and +60 mV, respectively, before application of clofilium, but they increased significantly to 530 ± 99 (*n* = 5, *P* < 0.02) and 3096 ± 584 nA (*n* = 5, *P* < 0.05) at –20 and +60 mV, respectively, after application of 100 µM clofilium.

Steady-state inactivation

Steady-state inactivation was studied by use of a standard double-pulse protocol. Measurements were made before and

Figure 3 Effects of clofilium (10, 100 µM) on the current of cloned transient outward-type K⁺ channels (RHK1) elicited from a holding potential of –60 mV. Depolarizing pulses of 250 ms in duration (+40 mV) were given at intervals of 20 s. (a) Original current tracings before (C) and during superfusion with 10 and 100 µM clofilium (10,100) and after washout (W). (b) Time courses of the effects on the *I*_{to} amplitude (●) and the rate of current inactivation (○).

4 min after superfusion with clofilium for each oocyte. In the presence of clofilium (100 μ M), when the depolarizing conditioning pulse was at -70 mV, the current amplitude during the second pulse did not change. However, the current was markedly enhanced when the conditioning pulse was at -55 mV (Figure 5a). Finally, we found that clofilium shifted the inactivation curve in the direction of depolarization (Figure 5b). In order to quantify the steady-state inactivation, the current amplitude of each second pulse was normalized to the control amplitude (no conditioning pulse and no drug) to give relative I , and plotted against the membrane potential of the conditioning pulse. For each individual cell, the equation of Boltzmann distribution was well fitted to the points and those parameters are shown in Table 1. $I_{\max}$ (relative) was 0.92–0.96 in the presence of 10–300 μ M clofilium. This indicates that clofilium does not change the amplitude of the maximum current obtained at large negative conditioning potentials (-70 to -80 mV). The slope factor, s , was 3.88 mV for control and did not change significantly after application of clofilium (Table 1). The mid-potential, V_h , was -61.13 ± 0.70 for the control conditions and it was shifted by clofilium in the direction of more positive potentials: -57.62 ± 0.82 and -50.87 ± 0.75 at 10 and 100 μ M, respectively. The effect of 300 μ M clofilium was similar to that of 100 μ M.

Effects of LY97119

LY97119(LY) is a desethyl tertiary ammonium derivative of clofilium, although clofilium is a quaternary ammonium, positively-charged compound. Figure 6 shows the effects of

Figure 4 (a) Original current tracings before (left) and after superfusion with 100 μ M clofilium (right). Currents were elicited by 250 ms depolarizing pulses from a holding potential of -60 mV to test potentials between -40 and $+60$ mV with 20 mV increments. (b) The peak amplitude of RHK1 current in the absence (○) and the presence of 100 μ M clofilium (●) in the above experiment was plotted against the depolarizing step potentials.

Figure 5 Effects of clofilium on the voltage-dependent inactivation of the cloned transient outward-type K⁺ channel (RHK1) current. (a) Current tracings during pulse 2 (P2, see the inset in (b) for pulse protocol) in the absence and presence of 100 μ M clofilium. The oocyte was held at -80 mV and stepped to -70 (left) or -55 mV (right) for 5 s and then stepped to $+30$ mV to activate the RHK1 current. The interpulse interval was 10 ms, and a double pulse was applied every 20 s. Leak and capacitive currents were not subtracted. (b) Graph indicating voltage-dependence of inactivation. The current amplitudes during P2 pulse plotted against membrane potentials of P1 are expressed as relative values to that during P2 pulse following P1 pulse at -80 mV in the absence of drug. Solid lines are drawn according to Boltzmann distribution: $I/I_{\max} = 1/(1 + \exp[(V - V_h)/s])$, where I is the relative current of P2 and V is the membrane potential of P1. The midpotential, V_h , and the maximum current, $I_{\max}$, were -61.1 mV and 0.98, respectively, for the control conditions (○, $n = 9$) and -51.0 mV and 0.97 in the presence of 100 μ M clofilium (●, $n = 4$). Each point represents the mean $\pm$ s.e.mean.

Table 1 Inactivation parameters for RHK1 channels before and after application of clofilium and LY97119

	$I_{\max}$	V_h (mV)	s (mV)	n
Control	0.99 ± 0.01	-61.13 ± 0.70	3.88 ± 0.12	9
Clofilium				
10 μ M	0.96 ± 0.07	$-57.62 \pm 0.82^*$	3.89 ± 0.18	4
30 μ M	0.92 ± 0.04	$-56.53 \pm 0.84^{**}$	3.81 ± 0.15	4
100 μ M	0.97 ± 0.06	$-50.87 \pm 0.75^{**}$	3.32 ± 0.18	4
300 μ M	0.93 ± 0.04	$-51.07 \pm 1.89^{**}$	3.65 ± 0.49	4
LY97119				
100 μ M	$0.72 \pm 0.05^{**}$	-61.90 ± 0.32	3.89 ± 0.23	5

The figures are parameters for the best fit curves of the equation: $I/I_{\max} = 1/(1 + \exp[(V - V_h)/s])$, where I is the peak amplitude of currents during P2, $I_{\max}$ is the maximum current, V_h is the midpotential and s is the slope factor. Before curve fitting, the currents were normalized by taking the maximum current ($P1 = -80$ mV) in the absence of drug as 1.0 for each oocyte. * $P < 0.02$. ** $P < 0.01$, unpaired t test.

Figure 6 Effects of LY97119 (LY) (10, 100 μ M) on the current of cloned transient outward-type K⁺ channels (RHK1) in oocytes injected with RHK1 cRNA. Currents were elicited by 250 ms depolarizing pulses (+40 mV) from a holding potential of -60 mV at intervals of 20 s. (a) Original current tracings before (C) and during superfusion with 10 and 100 μ M LY (10, 100) and after washout (W). (b) Time courses of effects on the I_{to} amplitude (●) and on the rate of current inactivation (○).

LY on the same system under the same conditions as those in the experiment shown in Figure 3 for clofilium. At a concentration of 10 μ M, LY accelerated inactivation of the current without having an effect on the current amplitude. Increasing the concentration to 100 μ M did not enhance the current amplitude unlike clofilium, but decreased it: 2630 ± 399 and 1110 ± 164 nA ($n = 6$, $P < 0.01$) before and after application of LY, respectively.

A large acceleration of current inactivation was observed as was seen with clofilium. The time courses of the onset and recovery were similar to those of the decrease in peak amplitude of currents. The time constant of current inactivation during +40 mV depolarizing pulses was 30.0 ± 0.9 ms ($n = 5$) before application of LY. After perfusion of LY (100 μ M), it decreased significantly to 18.0 ± 3.1 ms ($n = 5$, $P < 0.01$).

The effects of LY (100 μ M) on steady state inactivation are shown in Table 1. I_{max} was 0.72 for LY, indicating that LY suppressed the current amplitude also at a holding potential of -80 mV as well as at -60 mV. The slope factor, s , did not change upon application of LY, and there was no significant difference between the V_h values before and after perfusion with LY.

Discussion

Site of action of clofilium

Clofilium exhibited two distinct effects on I_{to} . One was an acceleration of current inactivation, and the other an augmentation of current amplitude when channels were maintained at a depolarizing holding potential.

The former effect is similar to its inhibitory effect on I_{to} of isolated cardiac cells (Castle, 1991) or on I_K of NG 108-15 cells (Reeve & Peers, 1992). Since this effect is not accom-

panied by modulation of the activation rate, clofilium acts on the activated state of channels and blocks the channel, or it accelerates conversion to the inactivated state from the open state. They reported that the onset of this effect was very slow and irreversible. In our oocyte experiments, where the environment surrounding the channels was different from that of cardiac cells, a partial and slow recovery from the effect was observed, but the onset of effect was also slow. On the other hand, LY, the analogue without a permanent positive charge, showed a faster onset and a stronger effect than clofilium. Therefore, the site of action is likely to be an intramembrane or intracellular site of the channel, as speculated by Castle (1991). Although clofilium has a permanent charge, it has a lipophilic nature as a whole. With a long exposure time, clofilium may be able to reach the binding site.

The second effect, an enhancement of the current, is considered to be elicited by clofilium bound to another site. Evidence supporting this second binding site is twofold: the time course of the onset of and recovery from the enhancement effect is different from that of the former effect, and the analogue LY showed only the former, inhibitory effect without the latter, enhancing effect. This binding site is probably extracellular, because the onset and washout of the clofilium effect was very fast in spite of the permanent charge carried by the clofilium molecule. Since the nitrogen of LY is mostly protonated in an aqueous environment ($pK_a = 9.3$), LY also has a positive charge in extracellular solution like clofilium. The difference between LY and clofilium is the presence of an ethyl group linked to the nitrogen in clofilium. Therefore, the extracellular site may recognize and accommodate the hydrophobic and bulky ethyl group.

Clofilium and channel inactivation

The clofilium-induced enhancement of the current occurs by a shift of the inactivation curve in the direction of more positive potentials. This is a parallel shift since clofilium does not shift the curve vertically. Therefore, at a holding potential of -80 mV, where no inactivation occurs, clofilium does not show the enhancement effect. However, under depolarized conditions, where the channels are partially inactivated, clofilium increases the current amplitude.

The inactivation curve of Figure 5, expressed by

$$I/I_{max} = 1 / \{1 + \exp[(V - V_h)/s]\}$$

is originally the equation of Boltzman's distribution, the ratio of the number of resting channels ([R]) in the total number of channels ([R] + [I]) in a steady state:

$$[R]/([R] + [I]) = 1 / \{1 + \exp[-(E_i - E_r)/RT]\}$$

where E_r and E_i are the energy levels of the resting and inactivated states, respectively. Therefore, the difference in energy level between the two states ΔE at a membrane potential V is

$$\Delta E = E_i - E_r = -RT(V - V_h)/s.$$

Under the control conditions, $s = 3.88$ mV, $V_h = -61.13$ mV, and $RT = 2.44$ kJ mol⁻¹ (21°C), the difference is -0.71 kJ mol⁻¹ at -60 mV, which means the channel favours the inactivated state a little more than the resting state. However, in the presence of 100 μ M clofilium, where most channels carry the bound clofilium because the effect was maximal at 100 μ M, ΔE increases to 5.75 kJ mol⁻¹ ($V_h = -50.87$ mV). This means that the binding of clofilium changes the stability of the respective conformations of the channel protein and makes the resting state more stable by 5.75 kJ mol⁻¹ than the inactivated state at -60 mV. Therefore, the number of channels in the resting state is much greater than that in the inactivated state. This would result in an increase in current when the depolarizing pulse is applied.

The increase in ΔE by clofilium is independent of the membrane potential:

$$\Delta E_{\text{drug}} - \Delta E_{\text{control}} = -RT (V_{h\text{control}} - V_{h\text{drug}})/s$$

Calculation yields 6.46 kJ mol^{-1} at $100 \mu\text{M}$ at all potentials. Therefore, even at a holding potential of -80 mV , clofilium modulates ΔE to the same extent as at -60 mV . However, at a holding potential of -80 mV , most of channels are in resting state even without drug, and therefore the same effect of clofilium on the channel protein does not result in the increase in measurable currents. The value 6.46 kJ mol^{-1} is not so large, compared to 19 kJ mol^{-1} of the stabilizing energy by one hydrogen bond (Lehninger, 1975).

This effect is unique and there is no other compound known to act in a similar manner. Bay K8644 also increases the current of Ca^{2+} channels, but it is not due to the shift of inactivation curve in the depolarizing direction. Bay K8644, as well as other dihydropyridines, shifts the curve in the hyperpolarizing direction (Sanguinetti *et al.*, 1986).

Physiological implications

Clofilium has two effects, an inhibitory one and an enhancement of I_{to} . Under depolarized conditions, because the enhancing effect is larger than the inhibitory one, the overall amount of current at a holding potential of -60 mV becomes larger in the presence of clofilium than in its absence (Figure 7). This means that the effects of clofilium on cells depends on the conditions.

Clofilium is well known as an antiarrhythmic drug. The mechanism has been explained by its inhibitory effect on I_{K} (Arena & Kass, 1988) and the following prolongation of the action potential duration. Compared to I_{K} , the role of I_{to} on the action potential has been poorly understood. Beuckelmann *et al.* (1993) showed that 4-aminopyridine inhibited I_{to} and increased action potential duration. However, the prolonged action potential duration may not be due to I_{to} inhibition because 4-aminopyridine is well known to inhibit I_{K} . On the other hand, Zhang *et al.* (1991) reported that 4-aminopyridine blocked I_{to} but shortened the action potential duration.

References

- ARENA, J.P. & KASS, R.S. (1988). Block of heart potassium channels by clofilium and its tertiary analogs; Relationship between drug structure and type of channel blocked. *Mol. Pharmacol.*, **34**, 60–66.
- BEAN, B.P. (1984). Nitrendipine block of cardiac Ca channels: High-affinity binding to the inactivated state. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 6388–6392.
- BEUCKELMANN, D.J., NABAUER, M. & ERDMANN, E. (1993). Alterations of K currents in isolated human ventricular myocytes from patients with terminal heart failure. *Circ. Res.*, **73**, 379–385.
- CASTLE, N.A. (1991). Selective inhibition of potassium currents in rat ventricle by clofilium and its tertiary homolog. *J. Pharmacol. Exp. Ther.*, **257**, 342–350.
- COLANSKY, T.J., FOLLMER, C.H. & STARMER, C.F. (1990). Channel Specificity in antiarrhythmic drug action. *Circulation*, **82**, 2235–2242.
- FOLANDER, K., SMITH, J.S., ANTANAVAGE, J., BENNETT, C., STEIN, R.B. & SWANSON, R. (1990). Cloning and expression of the delayed-rectifier I_{K} channel from neonatal rat heart and diethylstilbestrol-primed rat uterus. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 2975–2979.
- LEHNINGER, A.L. (1975). In *Biochemistry*, p.69. New York: Worth Publishers.
- REEVE, H.L. & PEERS, C. (1992). Blockade of delayed rectifier K currents in neuroblastoma x glioma hybrid (NG 108-15) cells by clofilium, a class III antidysrhythmic agent. *Br. J. Pharmacol.*, **105**, 458–462.
- SAKUTA, H., OKAMOTO, K. & WATANABE, Y. (1993). Antiarrhythmic drugs, clofilium and cibenzoline are potent inhibitors of glibenclamide-sensitive K currents in *Xenopus* oocytes. *Br. J. Pharmacol.*, **109**, 866–872.
- SANGUINETTI, M.C., KRATTE, D.S. & KASS, R.S. (1986). Voltage-dependent modulation of Ca channel current in heart cells by BayK8644. *J. Gen. Physiol.*, **88**, 369–392.
- TEN EICK, R.E., WHALLEY, D.W. & RASMUSSEN, H.H. (1992). Connections: heart disease, cellular electrophysiology, and ion channels. *FASEB J.*, **6**, 2568–2580.
- TSENG-CRANK, J.C.L., TSENG, G.N., SCHWARTZ, A. & TANOUYE, M.A. (1990). Molecular cloning and functional expression of a potassium channel cDNA isolated from a rat cardiac library. *FEBS Lett.*, **268**, 63–68.
- ZHANG, K., HARVEY, R.D., BARRINGTON, P.L., SCHACKOW, T.E., BASSETT, A.L. & TEN EICK, R.E. (1991). Transient outward current in normal and hypertrophied cat right ventricular myocytes. *Biophys. J.*, **59**, 559.

Figure 7 Total amount of RHK1 currents, which are the area under current tracings during 250 ms and expressed as relative values to the maximum current ($+60 \text{ mV}$ depolarization from a -80 mV holding potential in the absence of drug) for each oocyte. Control conditions from a holding potential of -80 (○) or -60 mV (△) and in the presence of $100 \mu\text{M}$ clofilium from a holding potential of -80 (●) or -60 mV (▲). Each point represents the mean $\pm$ s.e.mean of 5 experiments.

I_{to} determines the membrane potential of the plateau phase that has a profound influence on both Ca^{2+} currents and I_{K} during the action potential, and has a major role in the activity of cardiac cells. Therefore, it is of interest whether the effect of clofilium on a cloned I_{to} channel RHK1 is also observed in native cardiac I_{to} .

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