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Cellular Electrophysiology of Clofilium, a New Antifibrillatory Agent, in Normal and Ischemic Canine Purkinje Fibers

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Summary: Intracellular electrophysiological studies were performed on isolated canine cardiac tissues to investigate further the reported ability of clofilium (3×10^{-8}–10^{-6} M) to selectively increase action potential duration (APD) and refractoriness. In Purkinje fibers from normal dogs, clofilium did not influence (1) the rate of rise of the action potential ($\dot{V}_{max}$) elicited from normal or depolarized (10 mM potassium) resting potentials, (2) the $\dot{V}_{max}$ of premature potentials elicited during the repolarization phase of a previous action potential or (3) the rate of diastolic depolarization of spontaneously firing Purkinje fibers. The diastolic interval was altered by inserting a single premature impulse during diastole or by varying the basic cycle length. Clofilium (3×10^{-7} M) slightly reduced the time constant for the relation between diastolic interval and APD in concentrations that caused a maximal increase in APD of nonpremature impulses. In dogs subjected to occlusion of the left anterior descending coronary artery 48 hr before study, the APD of surviving Purkinje fibers was longer in the infarcted zone than in the normal zone. Clofilium (3×10^{-8} M) increased APD in both zones but more so in the normal area, thus reducing the disparity of APD between zones. Similarly, clofilium (3×10^{-8} and 3×10^{-7} M) increased the effective refractory period in both zones but more so in the normal area. The increase of APD and refractoriness in normal as well as depolarized or ischemic tissues in the absence of marked changes in $\dot{V}_{max}$ and conduction may decrease the likelihood of reentrant arrhythmias and underlie the antifibrillatory effects in anesthetized dogs.
Key Words: Clofilium—Action potential duration—Purkinje fibers—Reentry—Potassium current.

Clofilium (4-chloro-*N*,*N*-diethyl-*N*-heptyl-benzene butanaminium phosphate) selectively prolongs cellular action potential duration (APD) and effective refractory period (ERP) of isolated canine cardiac tissues obtained from normal dogs (1). In pentobarbital-anesthetized dogs, clofilium elevates the ventricular fibrilla-

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tion threshold, may permit electrically induced ventricular fibrillation to convert spontaneously to normal sinus rhythm without electrical counter shock (1), and reduces the defibrillation threshold (2).

The initial intracellular electrophysiologic studies of clofilium were performed on tissues from normal dogs. The present study was undertaken to extend these initial observations and to determine the effects of clofilium on ischemic tissues from dogs subjected to coronary artery occlusion or in tissues depolarized by solutions containing elevated potassium. Depolarized or ischemic cells may contribute to the occurrence of cardiac arrhythmias *in vivo* (3) and influence the response of cardiac tissues to antiarrhythmic agents (4,5). Also, since Purkinje fiber APD is critically dependent on the preceding diastolic interval (6,7), we determined the effect of clofilium on Purkinje fiber action potentials elicited at different basic cycle lengths and varying degrees of prematurity. A preliminary report of the data has been presented (8).

METHODS

Adult mongrel dogs weighing between 8 and 15 kg were anesthetized with sodium pentobarbital (30 mg/kg) and the hearts quickly removed. In most studies, the distal portions of the right or left bundle branches or free-running false tendons were impaled. In some studies the entire conducting system of the right or left ventricle was excised, and recordings were made from the area of the distal common bundle, the false tendon, the Purkinje fiber-muscle junction, and papillary muscle.

After dissection, tissues were pinned to the bottom of a 1.4 ml or 5 ml wax-lined bath and continuously superfused (5–6 ml/min) with modified Tyrode's solution having a pH of 7.4 and aerated with 95% O_2–5% CO_2. A Haake circulating heater maintained the tissue bath and superfusing solution at 35°C. The temperature was constantly monitored by a thermistor embedded in the wax. Unless stated otherwise, the solution contained the following ions (mEq/liter): Na^+, 156.7; K^+, 4.0; Mg^{2+}, 1.0; Ca^{2+}, 4.0; Cl^-, 145.9; $H_2PO_4^-$, 1.8; HCO_3^-, 18.0; glucose (5.5 mM) was also present.

The tissues were stimulated through bipolar, Teflon-coated, stainless-steel electrodes with square-wave pulses of 0.5 msec in duration at 1.5 times threshold voltage (usually 4–6 V). Unless stated otherwise, the basic cycle length of stimulation (S_1) was 1,000 msec. Tissues were allowed to reach a steady state by superfusing normal Tyrode's solution for at least 1.5 hr, during which time the action potential was continuously monitored to insure stability. Intracellular potentials were recorded with glass microelectrodes filled with 3 M KCl (5–20 MΩ, DC resistance) and connected to a high-impedance unity gain electrometer (W-P Instruments, model 725, 750 or M4-A). Electronic differentiation was used to obtain the maximum rate of rise of the action potential ($\dot{V}_{max}$). The output of this amplifier was linear for upstroke velocities up to 1,000 V/sec. Signals were displayed on a storage oscilloscope (Tektronix model 564) and recorded photographically. Action potential durations at 50, 70, and 95% of full repolarization (APD_{50}, APD_{70}, and APD_{95}), refractory periods, and $\dot{V}_{max}$ were also measured

with the aid of an on-line computer (DEC PDP 11/45, coupled to an analog/digital converter with a sampling interval of 50 μsec for 10 msec after the stimulus artifact and then 1 msec for the next 800 msec).

The effect of drugs on membrane responsiveness, defined as the relationship between the $\dot{V}_{max}$ of a premature impulse and the membrane potential from which the impulse arose (take-off potential), was determined by inserting premature stimuli (S_2, twice the voltage of S_1 impulses) during the repolarization phase of the action potential. The expression "h" defined as the ratio of $\dot{V}_{max}$ of the premature potential during repolarization to the peak value of $\dot{V}_{max}$ obtained at the maximum diastolic potential was determined before and after clofilium (10^{-6} M). The sigmoidal relationship between "h" and take-off potential was estimated as described by Bigger and Mandel (9). In other studies, the duration of premature impulses evoked during the latter stages of repolarization and during electrical diastole was measured as a function of the prior diastolic interval. The effect of frequency of stimulation on APD was determined by varying the cycle length from 370 to 1,400 msec. In preliminary studies, these cycle lengths fully covered the exponential relation between APD and cycle length.

Studies on Ischemic Tissues

Mongrel dogs of either sex weighing 12–16 kg were anesthetized with sodium thiopental (15 mg/kg, i.v.). Following intubation with a cuffed endotracheal tube, anesthesia was maintained with 1.5% halothane in oxygen using a closed-system anesthesia apparatus. The left anterior descending coronary artery was isolated at the level of the tip of the left atrial appendage (ca. 12–15 mm distal to the circumflex branch) and was occluded by the two-stage procedure of Harris (9a). Following surgery, morphine sulfate (1.5 mg/kg, s.c.), tobramycin (10 mg, i.m.), and sodium cefazolin (20 mg/kg, i.m.) were administered, and the dog was returned to a pen. Forty-eight hours later, dogs were anesthetized with pentobarbital (see above), the heart removed, and the anterior left bundle branch with its insertion into the anterior papillary muscle was isolated and superfused with Tyrode's solution.

The preparation was stimulated with a single bipolar electrode located near the origin of the bundle branch in the normal zone. A single recording microelectrode was used to impale cells at various sites along the conducting network in the visually normal and infarcted areas—the infarcted zone being grossly discolored and sharply delineated from the normal zone. Local ERP was determined by the following technique: one stimulating electrode was placed in the center of the ischemic endocardial zone and another in the normal zone, and a minimum of four cells at each site were impaled within several millimeters of the stimulating electrodes. A single premature stimulus (S_2) was introduced during the plateau phase and the S_1S_2 interval gradually lengthened until a propagated response was elicited. Local ERP was defined as the longest S_1S_2 interval at which a premature stimulus of twice S_1 voltage failed to conduct to the local recording electrode.

Clofilium phosphate (MW 437) was dissolved in water before dilution in Tyrode's solution. Our previous experiments had shown that a concentration of 3×10^{-8} M

prolonged APD by about half the maximum value and 3×10^{-7} M produced a maximal increase (1). Preliminary experiments conducted with intravenous [^{14}C]clofilium in intact animals did not show any correlation between blood and heart levels of radioactivity. Clofilium was superfused for at least 1 hr until the APD did not change by more than 5 msec over a 10 min period of observation. All values are expressed as means ± SEM. The significance of the difference between means was determined using a paired-sample t-test comparing the control values and the values obtained after drug exposure. Values of $p < 0.05$ were considered significant. Standard linear-regression analysis (10) was used to determine correlation coefficients, slope, and time constants for the relationship between diastolic interval and APD.

RESULTS

Effects on $\dot{V}_{max}$

We studied the effect of clofilium on the $\dot{V}_{max}$ of potentials elicited during the repolarization phase of the action potential (membrane responsiveness). A concentration of 10^{-6} M [about 75 times the EC_{50} for prolonging APD (1)] did not significantly alter the membrane responsiveness relation (Fig. 1). In addition, clofilium (3×10^{-7} M, $n = 7$) did not alter the conduction velocity of impulses conducted between two recording electrodes (2.0 ± 0.1 and 1.9 ± 0.2 m/sec before and after clofilium, respectively). In Purkinje fibers exposed to Tyrode's solution containing 10 mM potassium, clofilium (3×10^{-8} or 3×10^{-7} M) did not significantly alter $\dot{V}_{max}$, but it did slightly reduce amplitude at 3×10^{-8} M (though not at 3×10^{-7} M) and increase resting potential at 3×10^{-7} M (Table 1).

Automaticity

Purkinje fibers that were spontaneously automatic or fibers made automatic by 2 mM potassium and 10^{-6} M norepinephrine, hypoxia (95% N_2-5% CO_2) or ischemia (prior coronary occlusion) were exposed to 3×10^{-7} M clofilium for at least 40 min ($n = 15$, separate experiments). This concentration did not influence the rate of diastolic depolarization (9.2 ± 1.8 and 8.9 ± 1.8 mV/sec before and after clofilium, respectively). There was a small reduction in the rate of firing from 34 ± 5 to 30 ± 4 beats/min ($p < 0.05$) and a slight increase in the maximum diastolic potential from −87 ± 5 to −92 ± 5 mV ($p < 0.05$).

APD and Diastolic Interval

The relationship between the diastolic interval and APD was studied in two ways: (a) by interposing a single S_2 impulse during electrical diastole and late repolarization or (b) by changing the basic cycle length.

Figure 2 shows that at a basic cycle length of 1,000 msec, the APD_{95} of a premature impulse decreased as the S_1S_2 interval was reduced; the shortest S_1S_2 interval approximating the ERP of the cell. In the presence of clofilium (3×10^{-7} M), the shortest S_1S_2 interval that elicited a propagated response was increased by

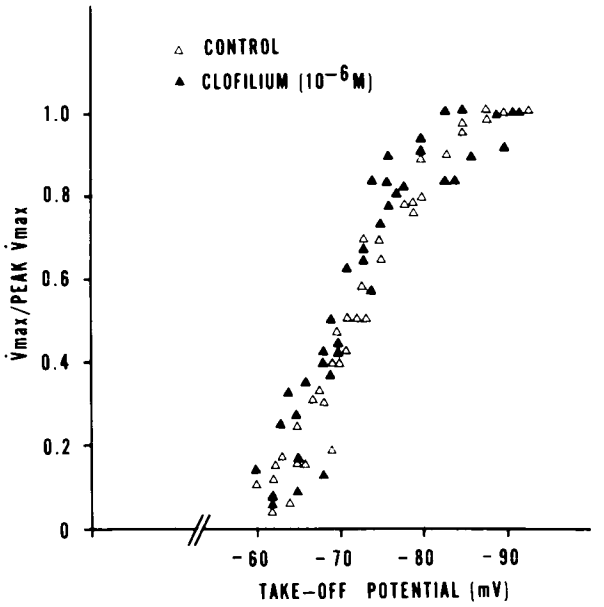

FIG. 1. Lack of effect of clofilium on membrane responsiveness. Ordinate: $\dot{V}_{max}$/peak $\dot{V}_{max}$ of the premature potential, where $\dot{V}_{max}$ occurs during the repolarization phase and peak $\dot{V}_{max}$ occurs at the maximum diastolic transmembrane potential. Abscissa: Membrane potential at which premature response is elicited. Shown are individual values for 4 experiments before and after superfusion with clofilium (10^{-6} M). The experimental values were fit to the expression $\dot{V}_{max}$/peak $\dot{V}_{max} = h = 1/[1+\exp(V_h - V_m)/d]$ using a nonlinear least-squares technique (10), where V_h = take-off potential at which h is one-half the maximum and where d is a constant determining the slope of the curve. Using these techniques, estimates of d before and after clofilium were 4.6 ± 0.3 and 4.4 ± 0.3, respectively. Corresponding values for V_h were −72.0 ± 0.2 and −70.3 ± 0.3 mV ($p < 0.05$). Calculated S-shaped curves based on the above equation were nearly identical and for clarity are not shown. Values for peak $\dot{V}_{max}$ before and after clofilium were 559 ± 39 and 534 ± 36 V/sec, respectively (not significantly different).

157 ± 16 msec (n = 7), which is similar to the 147 ± 10 msec increase in APD at the basic cycle length of 1,000 msec. Clofilium prolonged the APD_{95} of the earliest premature impulse by about the same percentage (38 ± 8) as the impulse elicited at the basic cycle length of 1,000 msec (34.0 ± 2.7). Plotting diastolic interval (S_1S_2 interval − APD_{95} of the S_1 impulse) against the $\log_e$ of the percentage decrease of APD_{95} (using APD_{95} at 1,000 msec as base line) demonstrated a monoexponential relationship that yielded an estimated time constant of 152 ± 7 msec (Fig. 3). In the presence of clofilium (3×10^{-7} M), this value was reduced to 130 ± 10 msec ($p < 0.05$).

Changing the diastolic interval by reducing the basic cycle length from 1,400 to 370 msec progressively shortened APD in the presence and absence of clofilium (3×10^{-7} M; Fig. 4). Both in absolute and percentage terms, clofilium increased APD_{95} less at shorter cycle lengths. For example, at a cycle length of 370 msec there was a 13.8 ± 2.1% increase in APD_{95} due to clofilium compared to a 28.0 ± 5.2% increase at a cycle length of 1,400 msec ($p < 0.01$). Plotting the diastolic interval against the $\log_e$ of the percentage decrease in APD_{95} (using the ADP_{95} of

TABLE 1. *Effect of clofilium on action potential parameters of Purkinje fibers partially depolarized by 10 mM potassium*

	Resting potential (mV)	Amplitude (mV)	$\dot{V}_{max}$ (V/sec)	APD_{50} (msec)	APD_{70} (msec)	APD_{95} (msec)
Control (16)	−89 ± 1	124 ± 1	586 ± 44	286 ± 8	328 ± 9	382 ± 8
10 mM K^+ (16)	−67 ± 2[c]	84 ± 2[c]	157 ± 20[c]	129 ± 7[c]	163 ± 6[c]	202 ± 5[c]
10 mM K^+ (8)	−67 ± 3	83 ± 3	186 ± 34	113 ± 9	150 ± 8	190 ± 6
10 mM K^+ + clofilium, 3×10^{-8} M (8)	−69 ± 3	79 ± 4[a]	146 ± 27	138 ± 11[b]	181 ± 9[b]	219 ± 8[b]
10 mM K^+ (8)	−68 ± 3	85 ± 2	149 ± 17	141 ± 9	177 ± 8	205 ± 9
10 mM K^+ + clofilium, 3×10^{-7} M (8)	−74 ± 3[a]	86 ± 2	143 ± 17	253 ± 23[c]	295 ± 21[c]	321 ± 27[c]

In a total of 16 experiments, Purkinje fibers studied at a cycle length of 1,000 msec were superfused with Tyrode's solution containing 10 mM K^+ for 41 ± 5 min (first grouping). Eight cells were then exposed to 3×10^{-8} M clofilium (middle grouping) and another 8 cells to 3×10^{-7} M clofilium (bottom grouping) until a steady state was reached (about 1 hr). Each impalement was maintained continuously throughout the control period, superfusion with high-K^+ solution, and during exposure to clofilium. Values are mean ± SEM.

[a] $p < 0.05$, paired t-test. Compared to corresponding values in 10 mM K^+ before clofilium.
[b] $p < 0.01$, paired t-test. Compared to corresponding values in 10 mM K^+ before clofilium.
[c] $p < 0.001$, paired t-test. Compared to corresponding values in control or 10 mM K^+ before clofilium.

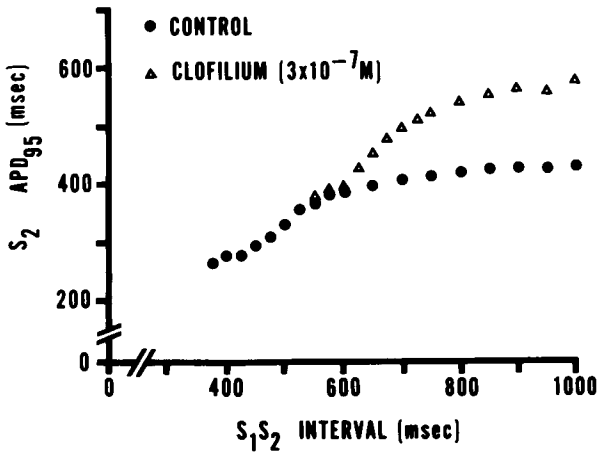

FIG. 2. Effect of clofilium (3×10^{-7} M) on the action potential duration at 95% of full repolarization (APD_{95}) of premature stimuli (S_2) elicited various times after the basic driven action potential (S_1). Ordinate: APD_{95} of premature impulse; abscissa: S_1S_2 interval. Basic cycle length of S_1 was 1,000 msec. Each point is the mean of 3–7 observations. The shortest S_1S_2 interval approximates the ERP of the cell. Values obtained following at least 1 hr superfusion with clofilium. SE bars removed for clarity.

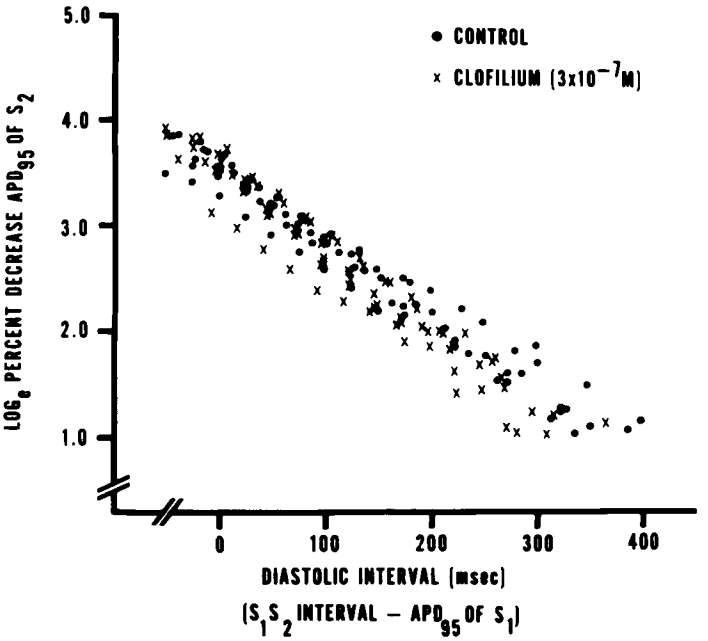

FIG. 3. Exponential relationship between the diastolic interval and action potential duration at 95% of full repolarization (APD_{95}) of a single premature impulse (S_2). Ordinate: $\log_e$ of APD_{95} of premature impulse expressed as a percent decrease of APD_{95} of the basic driven impulse (S_1) at 1,000 msec; abscissa: diastolic interval calculated as S_1S_2 interval minus the APD_{95} of S_1. Values are individual points from 66 observations in control and 61 observations in the presence of clofilium (3×10^{-7} M). Time constants: control, 152 ± 7 msec; clofilium, 130 ± 10 msec ($p < 0.05$). Correlation coefficients: control, 0.99; clofilium, 0.98. Values obtained after at least 1 hr superfusion with clofilium.

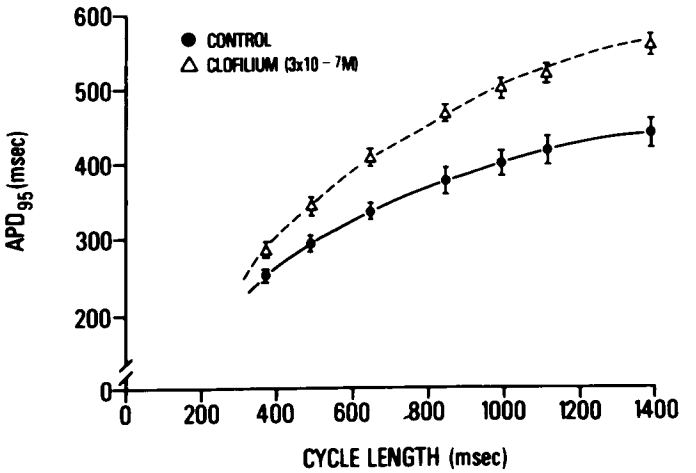

FIG. 4. Effect of clofilium (3×10^{-7} M) on action potential duration at 95% of full repolarization (APD_{95}) of impulses elicited at varying cyclic lengths. Ordinate: APD_{95} of driven impulse; abscissa: basic cycle length in msec. Each point is the mean ± SEM of six experiments. Values obtained after at least 1 hr superfusion with clofilium.

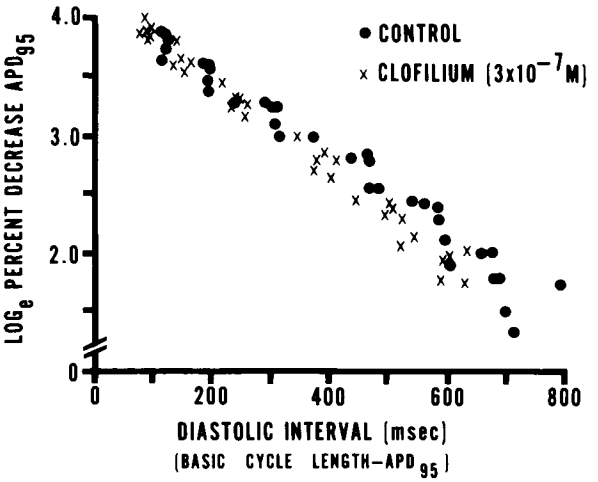

FIG. 5. Exponential relationship between the diastolic interval and action potential duration at 95% of full repolarization (APD_{95}) of impulses elicited at varying basic cycle lengths. Ordinate: $\log_e$ of APD_{95} of driven action potential expressed as a percent decrease from APD_{95} at 1,400 msec; abscissa: diastolic interval calculated as basic cycle length minus APD_{95}. Values are individual points from 36 observations in control and after clofilium (3×10^{-7} M). Time constants: control, 295 ± 12 msec; clofilium, 266 ± 7 msec ($p < 0.05$). Correlation coefficients: control, 0.98; clofilium, 0.99. Values obtained after at least 1 hr superfusion with clofilium.

the longest cycle length of 1,400 msec as base line) yielded a linear relation with time constants in the absence and presence of clofilium of 295 ± 12 and 266 ± 7 msec, respectively ($p < 0.05$; Fig. 5). The $\dot{V}_{max}$ of potentials elicited at the shortest basic cycle length of 370 msec in the absence and presence of clofilium was 427 ± 63 and 477 ± 40 V/sec, respectively (not significantly different).

APD of the Conducting Network

We determined the APD of cells within the conducting tissue of normal dogs from the origin of the left or right bundle branch to the termination in the subendocardium of the papillary muscle ($n = 14$, separate experiments). Figure 6 shows an example of such an experiment where APD_{95} is plotted against distance (in mm) from the stimulating electrode located near the origin of the left anterior bundle branch. The insertion of the bundle branch into the papillary muscle is indicated by the arrow. In this experiment, APD_{95} increased along the bundle branch, eventually reaching a maximum duration in an area 5–8 mm proximal to its insertion into the papillary muscle. Distally, the APD_{95} of the subendocardial Purkinje fibers decreased. However, this pattern was not a constant finding; in a few preparations no discernible area of maximum APD_{95} was noted. In all studies, however, clofilium (3×10^{-8} M) prolonged APD_{95} along the entire conducting network. In absolute terms, clofilium increased the Purkinje fiber APD_{95} relative to that of muscle. For example, before clofilium, the mean difference in APD_{95} between Purkinje fiber and muscle was 202 ± 13 msec; and after 3×10^{-8} and 10^{-7}

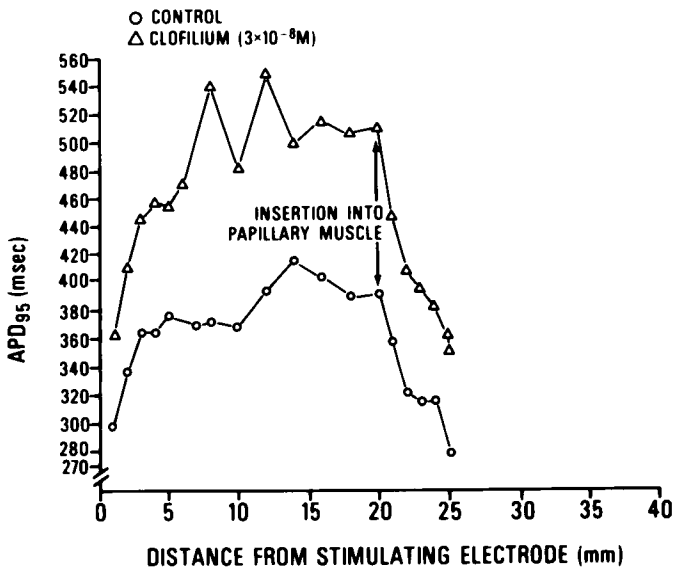

FIG. 6. Action potential duration at 95% of full repolarization (APD_{95}) of the left anterior bundle branch system before and after superfusion with clofilium (3×10^{-8} M). Ordinate: APD_{95} in msec; abscissa: distance in mm from stimulating electrode located near origin of bundle branch in septal wall. Arrows indicate insertion of free-running false tendon into body of left anterior papillary muscle. Clofilium superfused for 75 min.

M clofilium, this difference was 236 ± 21 and 290 ± 27 msec ($p < 0.01$, for both), respectively.

An example of the effect of clofilium on Purkinje fiber APD in dogs subjected to occlusion of the left anterior descending coronary artery 48 hr previously is shown in Fig. 7. In contrast to the results in normal dogs, APD_{95} progressively increased along the normal free-running false tendon, as well as into the distal infarcted area. Following superfusion with clofilium (3×10^{-8} M), APD_{95} was increased along the entire conducting network, although the increase was somewhat greater in the normal zone than in the infarcted area. A summary of the results for nine such experiments is shown in Table 2. Clofilium (3×10^{-8} M) increased APD_{95} in the normal zone by 107 ± 17 msec compared to 65 ± 15 msec in the ischemic zone ($p = 0.004$). Similarly, clofilium (3×10^{-8} M) increased APD_{70} by 87 ± 17 msec in the normal zone and 40 ± 8 msec in the ischemic zone ($p = 0.007$; data not shown).

In a separate series of experiments, the ERP of normal and ischemic tissues was studied before and after exposure to clofilium (3×10^{-8} M, $n = 9$; 3×10^{-7} M, $n = 8$). Before clofilium, the ERP in the ischemic zone was significantly longer than in the nonischemic area (361 ± 25 and 301 ± 14 msec, respectively, $p < 0.05$; Table 3). Clofilium (3×10^{-8} M) significantly increased ERP in both the ischemic and normal zones; the difference between the zones being reduced. After 3×10^{-7} M clofilium, an additional increase in ERP occurred in both zones, and the difference between ischemic and normal zones (434 ± 26 vs. 411 ± 32 msec, respectively)

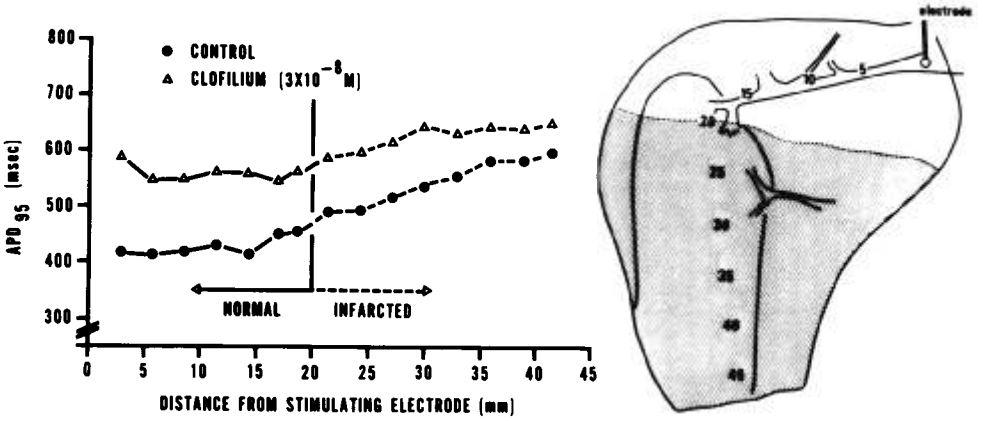

FIG. 7. Action potential duration at 95% of full repolarization (APD_{95}) of the left anterior bundle branch system in a dog subjected to occlusion of the left anterior descending coronary artery 48 hr previously. Ordinate: APD_{95} in msec; abscissa: distance in mm from stimulating electrode located as shown near the origin of the free-running strand in the normal zone. Shading denotes the infarcted area. Clofilium (3×10^{-8} M) superfused for 1 hr.

was further reduced. The lack of effect of clofilium on the ERP/APD ratio at APD_{70} and APD_{95} in both normal and ischemic zones is shown in Table 4. Moreover, these ratios in both normal and ischemic tissues before clofilium were similar. Clofilium had no significant effect on resting potential, amplitude, or $\dot{V}_{max}$ of cells in the ischemic area. In the normal area, the only significant drug effect noted (3×10^{-8} M) was a reduction in $\dot{V}_{max}$ from 431 ± 25 to 363 ± 23 V/sec ($p < 0.05$, $n = 16$).

TABLE 2. *Effect of clofilium on action potential duration at 95% of full repolarization (APD_{95}) in normal and infarcted regions of the left anterior ventricular conducting network*

	APD_{95} (msec)	
	Normal zone	Infarcted zone
Control ($n = 9$)	388 ± 16	471 ± 16
Clofilium, 3×10^{-8} M ($n = 9$)	494 ± 21	536 ± 25
Increase from control	107 ± 17[a]	65 ± 15[a]

Dogs were subjected to coronary artery ligation 48 hr before removing the heart. Values are mean APD_{95} (± SEM) of at least 10 sites impaled within the center of the visually normal or ischemic zones before and after superfusion with clofilium for at least 1 hr.

[a] $p < 0.01$, Student's t-test for paired data comparing values before and after clofilium in each zone.

TABLE 3. *Effect of clofilium on effective refractory period in normal and ischemic canine Purkinje fibers*

	Effective refractory periods (msec)		
		Clofilium	
	Control (n = 9)	3×10^{-6} M (n = 9)	3×10^{-7} M (n = 8)
Normal zone	301 ± 14	359 ± 24[b]	411 ± 34[b]
Ischemic zone	361 ± 25[a]	414 ± 36[b]	434 ± 26[c]

Tissues obtained from dogs 48 hr following coronary artery occlusion (see Methods). Values are means ± SEM.

[a] Significantly greater than corresponding value in normal zone: $p < 0.05$, paired t-test.

[b] Significantly greater than corresponding control value before clofilium: $p < 0.01$, paired t-test.

[c] Significantly greater than corresponding control value before clofilium: $p = 0.001$, paired t-test.

DISCUSSION

These results extend and confirm initial findings concerning the electrophysiological specificity of clofilium (1). Clofilium prolongs APD and refractoriness in concentrations that have little if any effect on the upstroke of the action potential, membrane resting potential, or rate of diastolic depolarization.

Lack of Effect on $\dot{V}_{max}$

The following observations suggest that under our experimental conditions, clofilium does not change $\dot{V}_{max}$: (a) the $\dot{V}_{max}$ of premature impulses elicited during

TABLE 4. *Effect of clofilium on the ratio of cellular effective refractory period (ERP) and action potential duration (APD)*

		Clofilium	
	Control (n = 9)	3×10^{-6} M (n = 9)	3×10^{-7} M (n = 8)
Normal zone			
ERP/APD_{70}[a]	0.927 ± 0.023	0.929 ± 0.032	0.893 ± 0.028
ERP/APD_{95}	0.769 ± 0.017	0.768 ± 0.026	0.752 ± 0.029
Ischemic zone			
ERP/APD_{70}	0.930 ± 0.047	0.965 ± 0.067	0.911 ± 0.048
ERP/APD_{95}	0.709 ± 0.031	0.705 ± 0.033	0.682 ± 0.028

Values following clofilium were obtained at steady state (about 1 hr after drug addition) and are expressed as means ± SEM of the number of observations in parentheses.

[a] APD measured at 70% full repolarization (APD_{70}) or 95% of full repolarization (APD_{95}).

or after repolarization was unchanged (Fig. 1); (b) $\dot{V}_{max}$ of action potentials elicited from depolarized membrane resting potentials (Table 1) was unaffected; (c) $\dot{V}_{max}$ was unchanged by clofilium at high stimulation rates; and (d) the ERP/APD ratio in normal and ischemic tissue was similar before and after clofilium (Table 4). Since under most circumstances $\dot{V}_{max}$ represents a valid index of the maximum sodium current during the upstroke (11,12), we conclude that in the concentrations studied, clofilium has little if any effect on the availability or kinetics of the rapid sodium channel.

Prolonging Purkinje fiber action potentials without affecting the kinetics of the sodium channel might allow more time for previously activated sodium channels to recover from inactivation, thereby *increasing* the experimentally derived membrane responsiveness relationship (12). However, the membrane responsiveness curve was not altered by clofilium (Fig. 1). Since recovery from inactivation begins after the membrane has repolarized to about −45 mV (13), the reopening of inactivated sodium channels may simply be delayed by the additional time the membrane remains depolarized under the influence of clofilium. Because the removal of inactivation is relatively rapid, especially at potentials more negative than about −75 mV (14), selective prolongation of APD would not be expected to influence the membrane responsiveness relation, since $\dot{V}_{max}$ should simply follow the steady-state inactivation variable (h_∞) in the absence or presence of drug.

Effects on Repolarization

Due to the inward rectifying property of cardiac membranes at plateau potentials, small changes in net current flow can cause marked alterations in the time course of repolarization of the action potential (15). Considering the multiplicity of currents flowing during the plateau and repolarization phase of the action potential, it is difficult to characterize the specific conductance(s) altered by drugs without voltage-clamp data (for review, see refs. 16–18). Nevertheless, sufficient information is available on the mechanism(s) of repolarization, especially in Purkinje fibers (15,19–22), to allow some speculation as to the membrane current(s) possibly affected by clofilium.

The i_{X1} is a time- and voltage-dependent outward current activated at plateau potentials that is thought to be the major current responsible for terminating the plateau of Purkinje fiber action potentials (15). Clofilium might increase APD by selectively interfering with either or both the activation or deactivation of this current. At plateau potentials, i_{X1} activates slowly (7); and in short action potentials, e.g., ventricular muscle or Purkinje fibers stimulated at high rates, insufficient time may elapse for i_{X1} to fully activate. If clofilium does interfere with the fully activated i_{X1}, this may partly explain why clofilium is somewhat less active in Purkinje fiber preparations stimulated at high frequency (Fig. 4) and in muscle cells or cells exposed to lidocaine (8). However, in preparations with action potentials shortened by high potassium concentrations (Table 1), clofilium increased APD to about the same degree as in normal fibers (1).

After activation, i_{X1} exponentially deactivates during diastole and is largely responsible for the interval-duration relation in Purkinje fibers (6,7,22). We found

that the interval-duration relation determined by premature impulses or by changing the basic cycle length could be fitted by an exponential function (Figs. 3 and 5) with time constants corresponding to the deactivation of i_{X1} (50–100 msec) at diastolic potentials (7). Clofilium had a small but statistically significant effect on the interval-duration relationship, so that at a given diastolic interval the percentage decrease of APD (compared to the APD at the longest diastolic interval) was slightly less than in control. We conclude that even in relatively high concentrations (3×10^{-7} M), clofilium may only slightly alter the deactivation kinetics of i_{X1}.

Like clofilium, reducing extracellular calcium (23) or potassium (24,25) concentration markedly increases Purkinje fiber APD, an effect thought to be mediated by a reduction in the time-independent potassium conductance (gK_1). Moreover, lowering extracellular calcium concentration has little or no effect on the interval-duration relation or resting membrane potential (26,27). Therefore, clofilium may—perhaps via a calcium-dependent mechanism (21,28)—reduce gK_1. Against this possibility, however, is the lack of change in resting potential, maximum diastolic potential, or rate of phase IV depolarization.

Effects on Ischemic Tissue

The electrophysiological effects of antiarrhythmic agents in ischemic or infarcted tissue may differ considerably from those in normal tissues (5,29,30). Previous studies have shown that Purkinje fiber action potentials are prolonged in ischemic tissues taken from dogs 24–48 hr following occlusion of a coronary artery (31,32). The disparity of APD between such prolonged cells and normal tissues could facilitate the occurrence of reentry (5,33). Clofilium increases the APD of Purkinje fibers in both the ischemic and normal zones (Table 2, Figs. 6 and 7). However, the prolongation was greater in the normal zones, which caused a small but significant decrease in disparity between zones. The ERP was also increased in both the ischemic and normal zones but slightly more in the normal zones (Table 3). Since clofilium did not alter the ERP/APD in ischemic or normal tissues from dogs subjected to coronary artery occlusion (Table 4), we conclude that the effect on refractoriness in ischemic, as in normal tissue (1), is due solely to the prolongation of APD. Although the small decrease in disparity between the normal and ischemic zones could reduce the likelihood of reentry (5), the increase in the absolute level of refractoriness in the normal as well as ischemic tissues may also be important.

Relation of Cellular Electrophysiological Effects to Potential Mechanism(s) of Antifibrillatory Activity

By prolonging APD and thus absolute refractoriness in normal and ischemic tissue without slowing conduction, the likelihood of initiating or sustaining reentry should be reduced (34–36). Since prolonging cellular refractoriness is the only direct electrophysiological effect of clofilium as yet detected, it is reasonable to suggest that this effect may underlie the antifibrillatory activity of clofilium dem-

onstrated in anesthetized dogs under nonischemic conditions (1,2). Within the peripheral Purkinje fiber network of nonischemic tissues, areas of maximum APD may act as a "gate" to limit the propagation of premature impulses (37,38). We found gate areas in some but not all preparations (Fig. 6). By enhancing refractoriness throughout the conducting network, as well as the gate area, clofilium may enhance the protective function of the distal gating system (39) and prevent propagation of premature impulses either distal or proximal to the gate area. By enhancing the APD, clofilium might allow the supernormal period to occupy proportionally less of the APD, which would also contribute to reduced excitability (40).

Drugs that can selectively prolong action potential duration and refractoriness have been termed "class III" antiarrhythmic agents (18,41,42). Examples of other agents that possess this property include bretylium, amiodarone, and sotalol (18,42,43). Clofilium may therefore be considered an example of a class III agent possessing high potency and selectivity.

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