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Electrophysiologic Effects of Verapamil on Cells Bordering Healed Myocardial Infarction in the Cat[1] (41513)

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Abstract. Verapamil (1 μg/ml) completely abolished a population of markedly depressed action potentials of cells (BZ-1) in border zones around healed infarcts. Even at higher concentrations, verapamil had no such inhibitory effect on other border zone cells (BZ-2), normal and central infarct zones cells. BZ-1 cells demonstrated a severely depressed $\dot{V}_{max}$ (<20 V/sec), accompanied by partial depolarization, while BZ-2 cells were characterized by abbreviated duration. Verapamil only significantly depressed $\dot{V}_{max}$ of BZ-1 cells. Action potential amplitude and action potential duration at 50% repolarization of all cells were abbreviated by verapamil. These results indicate slow inward currents may be involved in the genesis of action potentials in BZ-1 cells.

We recently reported significant action potential abnormalities in border zone ventricular muscle cells after healing of myocardial infarction in cats (1). Border zone cells are often depolarized and demonstrated a range (5.0–115.0 V/sec) in reduction of maximum rate of action potential upstroke velocity ($\dot{V}_{max}$). Slowing of $\dot{V}_{max}$ may be due to partial inactivation of a rapid Na^+-dependent current, activation of a slow inward current, or some combination of these currents (2–4). Verapamil exerts inhibitory action on the slow inward current across the myocardial membrane (5, 6). In the present study, we used verapamil to estimate the contribution of the slow channel to the generation of action potentials with depressed $\dot{V}_{max}$ in cells bordering healed myocardial infarction scars.

Materials and Methods. Acute myocardial infarction was created in 12 cats by single-stage ligation of two or three distal tributaries of the left coronary system (7). Surviving animals were maintained for 2–7 months. On the day of terminal studies, the heart was removed and dissected in cool, oxygenated, modified Tyrode's solution (composition in m*M*: NaCl = 129, $NaHCO_3$ = 20, dextrose = 5.5, KCl = 4.0, NaH_2PO_4 = 1.8, $MgCl_2$ = 0.5, $CaCl_2$ = 2.7). The atria and right ventricle were removed and the left ventricle was opened by an incision through its free wall between the two papillary muscles. The mitral valve was removed and the aortic ring opened. Preparations of left ventricle were mounted endocardial surface up in a lucite tissue bath and superfused with warmed (37°) Tyrode's solution equilibrated with 95% O_2–5% CO_2 (7).

Driving stimuli at a cycle length of 800 msec were delivered to the left bundle branch (unless noted elsewhere) through Teflon-coated silver wire electrodes, 0.01 in. in diameter. Pulse duration was 3 msec and current intensity was 1.5 times late diastolic threshold. While visible scars were almost always present over the infarcted area, surface electrograms, recorded through silver wire electrodes, were used to delineate areas of electrophysiologic abnormality (7).

Following identification of endocardial

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areas with abnormal surface electrogram, transmembrane potentials were recorded from ventricular muscle cells overlying and bordering healed myocardial infarction scars and from normal areas in the same heart, using standard microelectrode techniques (8). The glass microelectrodes filled with 3 *M* KCl (10–30 MΩ resistance) were connected through Ag–AgCl junctions to electrometers with input capacity neutralization (Bioelectric NF-1); their outputs were displayed on oscilloscope and recorded on film. Grids (6–12 mm^2) were constructed to provide a consistent sampling technique. Action potential characteristics were measured from the photographic records. $\dot{V}_{max}$ was electronically differentiated using a calibrating signal of known voltage and duration (9).

After random sampling, 3–10 representative cells from normal, infarct, and border zones in which continuous impalement could be maintained were selected for drug studies. An equilibration period of at least 60 min was allowed before initiating exposure to *l*-verapamil (Knoll). The data reported here represent the effects of drugs on maintained impalements of single ventricular muscle cells. For comparisons of the differences between mean data from central infarct, border and normal zones, an analysis of variance was employed (10). Comparisons of sample means between pretreatment and treatment of verapamil were made, employing the Student's *t* test for paired data (11).

Results. Action potentials with normal configuration were recorded from uninfarcted zones of the coronary-ligated hearts, while cells overlying the healed infarct were characterized by action potentials with prolonged duration (Table I). Border zone (~1-mm region surrounding the infarct) cells generated action potentials with varying degrees of depression of $\dot{V}_{max}$. Two distinct populations of border zone (BZ) cells became evident during exposure to verapamil. Action potentials of BZ-1 cells were completely abolished within 30 min exposure to 1 μg/ml verapamil despite stimulation at increased intensity, 0.5 mm from the recording site. These

TABLE I. COMPARISON OF THE ACTION POTENTIAL CHARACTERISTICS OF STIMULATED VENTRICULAR MUSCLE[a] FIBERS AT NORMAL, BORDER, AND INFARCT ZONES[b]

	Resting potential (−mV)	Action potential amplitude (mV)	APD_{50} (msec)	APD_{90} (msec)	$\dot{V}_{max}$ (V/sec)
NZ cell (8)[c]	82.3 ± 3.6[d]	103.4 ± 3.8	88.4 ± 7.2	128.7 ± 5.0	143.8 ± 23.8
IZ cell (8)	83.1 ± 2.4	108.1 ± 3.3	113.5 ± 6.8*	175.6 ± 10.3*	139.8 ± 15.9
BZ-1 cell (5)	51.5 ± 4.1*,†	57.5 ± 4.8*,†	60.8 ± 10.1*,†	121.1 ± 20.4†	11.4 ± 2.3*,†
BZ-2 cell (7)	66.0 ± 1.8*,†,‡	80.3 ± 4.7*,†,‡	45.2 ± 5.5*,†,‡	82.3 ± 9.6*,†,‡	82.2 ± 10.7*,†,‡

[a] Preparations were stimulated at a cycle length of 800 msec.
[b] NZ, normal zone; IZ, central infarct zone; BZ-1, border zone type 1; BZ-2, border zone type 2; APD_{50}, action potential duration at 50% repolarization; APD_{90}, action potential duration at 90% repolarization; $\dot{V}_{max}$, maximum rate of rise of phase 0 depolarization.
[c] Number of cats in parentheses; 3–10 cells per category (NZ, IZ, BZ-1, BZ-2) per cat.
[d] Data expressed as mean ± SE.
* Significant differences from normal, via analysis of variance ($P < 0.05$).
† Significant differences from central infarct, via analysis of variance ($P < 0.05$).
‡ Significant differences from BZ-1, via analysis of variance ($P < 0.05$).

verapamil-sensitive BZ-1 cells demonstrated a severely depressed $\dot{V}_{max}$ (<20 V/sec), accompanied by partial depolarization (Table I). In contrast, action potentials of the other border zone cells (BZ-2), as well as those recorded from normal and central infarct zones cells, were never abolished even at higher concentrations of verapamil. Action potentials of BZ-2 cells were characterized by abbreviated durations (both APD_{50} and APD_{90}) and moderate depression in $\dot{V}_{max}$ (Table I). During exposure to 1.0 μg/ml verapamil, only action potential amplitude (78 to 71 mV, −9%, n = 4, $P < 0.05$) and duration (APD_{50}, 47 to 35 msec, −26%; APD_{90}, 96 to 69 msec, −28%, n = 4, $P < 0.05$) were significantly altered in BZ-2 cells.

A lower concentration of verapamil (0.1 μg/ml) significantly depressed $\dot{V}_{max}$ (13 to 3 V/sec, −77%, n = 3, $P < 0.05$), action potential amplitude (59 to 33 mV, −44%, n = 3, $P < 0.05$), and shortened action potential duration (APD_{50}, 66 to 49 msec, −26%; APD_{90}, 134 to 109 msec, −19%, n = 3, $P < 0.05$) of the slowly rising action potentials of BZ-1 cells (Fig. 1a). In contrast, neither $\dot{V}_{max}$ nor action potential amplitude of BZ-2 cells was affected by 0.1 μg/ml verapamil; this drug concentration only further abbreviated the short action potential duration of BZ-2 cells (APD_{50}, 41 to 32 msec, −22%; APD_{90}, 82 to 67 msec, −18%, n = 3, $P < 0.05$) (Fig. 1b).

Verapamil (0.1 and 1.0 μg/ml) had no effect on action potentials of cells in uninfarcted areas. However, action potential amplitude and APD_{50} of such normal zone cells were significantly reduced by 2.0 μg/ml verapamil (102 to 98 mV, −4%, 90 to 77 msec, − 14%, n = 6, $P < 0.05$) (Fig. 2a). As in the normal zone cells, action potentials of central infarct zone cells were not affected by 0.1 μg/ml verapamil. However, 1.0 μg/ml verapamil significantly shortened APD_{50} of central infarct zone cells by 21% (122 to 96 msec, n = 4, $P < 0.05$). In addition to the reduction of action potential amplitude (109 to 100 mV, −9%, n = 6, $P < 0.05$) and shortening of APD_{50} (113 to 74 msec, −35%, n = 6, $P < 0.05$), 2.0 μg/ml verapamil significantly shortened APD_{90} (181 to 141 msec, −22%, n = 6, $P < 0.05$) of central infarct zone cells (Fig. 2b).

Discussion. The absence of a significant verapamil effect on $\dot{V}_{max}$ of normal and central infarct zones ventricular muscle cells is in keeping with its minimum effect

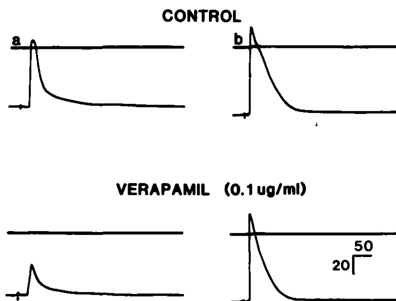

FIG. 1. The effects of 0.1 μg/ml verapamil on the action potential characteristics of ventricular muscle cells from BZ-1 (A) and BZ-2 (B) cells in the border zone. After 60 min superfusion with verapamil, the slowly rising action potential of BZ-1 cell was severely depressed and remained depressed despite increased stimulus intensity (10×), 0.5 mm from the recording site. In contrast, this concentration of drug only abbreviated the already short action potential duration of BZ-2 cell.

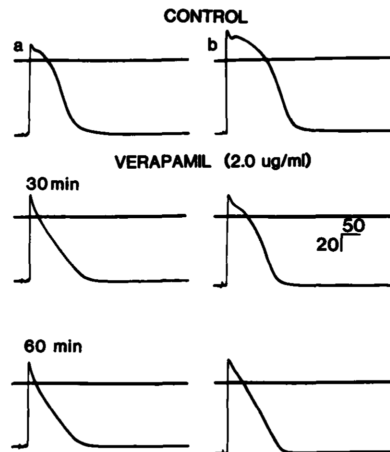

FIG. 2. The effects of 2.0 μg/ml verapamil on the action potential characteristics of ventricular muscle cells from the normal zone (A) and central infarct zone (B). After 30 and 60 min superfusion with verapamil, action potential amplitude and APD_{50} of both normal and central infarct zones cells were significantly reduced. Note the additional shortening of APD_{90} by verapamil monitored in central infarct zone cell.

on the fast Na^+ current in cat myocardium (5). This is consistent with previous reports that verapamil did not change $\dot{V}_{max}$ of normal ventricular cells (3, 12). We also observed that verapamil produced a significant decrease in action potential amplitude from normal and central infarct zones cells. A similar reduction in action potential amplitude by verapamil was documented in normal canine ventricular muscle (13) and it may be that the drug inhibits the Ca^{2+} current which contributes to the action potential overshoot (14).

The shortening APD_{50} of normal and central infarct zone cells and BZ-2 cells by verapamil is consistent with previous reports (15, 16) and can be attributed to a reduction in slow inward plateau current. However, we observed a reduction in APD_{90} of BZ-2 and central infarct zone cells, while that of the normal zone remained unchanged when exposed to verapamil. An alteration of the time-independent plateau current by verapamil (6), in addition to its other effects (a reduction of the time-dependent inward current and a voltage shift of slow outward current), could account for its shortening of APD_{90} of BZ-2 and central infarct zone cells.

The present study shows that in healed infarction myocardium, the electrophysiologic response of muscle cells to verapamil varies even within the border zone. Exposure to verapamil abolished a population of markedly depressed action potentials of cells (BZ-1) in border zones. However, even at higher concentrations of verapamil, action potentials of other border zone cells (BZ-2), as well as that of normal and central infarct zones were never abolished. This suggests that slow inward currents may play an important role in the genesis of action potentials in a border zone population subset. Verapamil-sensitive action potentials have been previously demonstrated in aneurysmal tissues up to 25 months after myocardial infarction in humans (17) and in infarcted tissues 24 hr after coronary ligation in the dog (18). However, in our feline model of healed myocardial infarction, verapamil-sensitive action potentials were most evident in the border zone.

The lack of verapamil effect on $\dot{V}_{max}$ and the persistence of electrical activity of BZ-2 cells in the presence of verapamil suggests that these action potentials were not slow channel dependent. Although a defect in the conductance of the fast Na^+ current is a possible mechanism, we cannot be sure of the ionic nature of the slightly depressed action potentials of BZ-2 cells.

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