Diltiazem Ameliorates Contractile Dysfunction Without Shortening of Action Potential Duration in Ischemic Heart of Anesthetized Dogs

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ABSTRACT—The purpose of the following experiment was to determine whether amelioration of myocardial contractile dysfunction by diltiazem is mediated by shortening of monophasic action potential duration (MAPD) during myocardial ischemia in anesthetized dogs. Diltiazem improved regional contraction during reperfusion after 10-min occlusion. The shortening of MAPD and increase in [K+], were blunted by treatment with diltiazem. These results suggest that shortening of action potential duration during myocardial ischemia is unlikely to be a reason for the amelioration of contractile dysfunction.

Keywords: Diltiazem, Ischemic heart, Action potential duration

Diltiazem, an L-type calcium channel blocker, is known to prevent ischemic arrhythmia during coronary artery occlusion (1). Our laboratory has demonstrated that the extracellular potassium concentration ([K+]e) accumulation is delayed by diltiazem in isolated guinea pig hearts (2, 3). Such a reduction of [K+]e by diltiazem may account for its antiarrhythmic action. However, diltiazem has been shown to have a beneficial effect on regional contractile dysfunction (myocardial stunning) after onset of reperfusion (4). Previously, this cardioprotective effect of diltiazem was postulated to be due to inhibition of Ca2+ influx via voltage-dependent Ca2+ channels. If this was the case then, diltiazem would shorten action potential duration, which might be arrhythmogenic cause. This seems paradoxical because of the known antiarrhythmic action of diltiazem. To date, it has not been fully determined to what extent the same dose of diltiazem can change action potential duration and regional contractile function in the same ischemic preparation. A direct comparison between these parameters may reveal the role of action potential duration on the cardioprotective effect of diltiazem. Therefore, we concurrently examined the effects of diltiazem on regional contractile function, monophasic action potential duration (MAPD) and [K+]e, in ischemic heart of anesthetized dogs.

Adult mongrel dogs of both sexes weighing 7 – 12 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Animal experiments were designed and conducted in compliance with the “Guiding Principles for the Care and Use of Laboratory Animals” approved by The Japanese Pharmacological Society. After tracheotomy, animals were artificially ventilated using a volume-cycled ventilator. A polyethylene catheter was inserted into the right femoral artery for measurement of systemic blood pressure (BP) and one inserted in the left femoral vein for administration of diltiazem. After left thoracotomy, a pressure tip transducer (3 spc-350; Millar, Houston, TX, USA) was inserted into the left ventricular cavity via the left atrium for measurement of left ventricular pressure (LVP) and the first derivative of LVP with respect to time (dP/dt) (EQ-601G; Nihon Kohden, Tokyo). The left anterior descending coronary artery (LAD) was dissected distally to the first diagonal branch, and a silk ligature was placed loosely around the vessel for occlusion. An electromagnetic flowmeter probe (Type FI, Nihon Kohden) was also placed distal to the ligature to measure coronary blood flow (CBF). A Doppler transducer (Crystal Biotec, Hopkinton, MN, USA) was attached to the epicardium in the ischemic bed, and the excursion of myocardium was measured at a depth 4.0 – 5.0 mm below the epicardial surface. End-diastolic wall thickness (EDT) and end-systolic wall thickness (EST) were determined, and percentage of wall thickening (thickening fraction, TF) during systole was calculated as (EST – EDT / EDT) × 100 (5).
prepared with valinonycin and a tridodecylamin matrix membrane, respectively, according to our previous method (6). Extracellular pH (pH$_e$) was calculated as pH = $-\log[H^+]$. A pair of ion-sensitive electrodes was perpendicularly inserted into the left ventricular myocardium at a depth of 4.5 mm from the ischemic epicardial surface. The site of the ion-sensitive electrodes was located 15 – 20 mm downstream from the diverging point on LAD and at a distance of 10 mm from the diagonal branch. Cardiac monophasic action potential was recorded on the ischemic epicardial surface using a bipolar concentric Ag/AgCl electrode. The MAPD was recorded <5 mm apart from the location of the paired ionic electrodes. This distance was necessary to avoid any influence of suction through an MAPD electrode on the ischemic measurement. We previously performed a feasibility study to eliminate the possibility that this distance might lessen the validity of the simultaneous measurements of MAPD and ion concentrations (7). MAPD was measured at 60% repolarization.

Ten animals were divided into control (n = 5) and diltiazem-treatment (n = 5) groups. LAD was occluded for 10 min and subsequently reperfused for 120 min. Diltiazem (20 μg/kg per hour) was injected intravenously 10 min prior to occlusion, which was stopped before the onset of reperfusion. All values were expressed as the mean ± S.E.M. Differences between the two groups were compared using two-way ANOVA with repeated measurements and Fisher’s least significant difference test. An unpaired t-test was used for comparison of values in the control group with those in the diltiazem-treatment group.

As shown in Table 1, there was no significant change in baseline value of BP, heart rate (HR), LVP, dP/dt$_{max}$, TF and CBF between the control and diltiazem-treatment group. In the diltiazem-treatment group, BP was significantly decreased during the 8-min occlusion and the 10-min reperfusion compared with the control value, indicating hypotensive effects of this compound, generally considered to be due to Ca$^{2+}$ channel blockade.

In both the control group and diltiazem-treatment group, coronary occlusion significantly decreased TF, an index of contractile function, at the ischemic region without changing HR, LVP and dP/dt$_{max}$. Incidence of arrhythmia in both groups was not observed during ischemia and reperfusion. In the control group, TF during 10-min reperfusion transiently rebounded but was again significantly reduced at 120 min after onset of reperfusion. In the presence of diltiazem, TF was significantly recovered at 120 min after onset of reperfusion. This suggests that diltiazem possesses a beneficial effect on cardiac function after ischemia.

The time courses for MAPD, [K$^+$], and pH$_e$ of the two groups are summarized in Table 2. In the control group, coronary artery occlusion significantly increased [K$^+$], decreased pH$_e$ and shortened MAPD. These results are consistent with previous findings that [K$^+$], and extracellular H$^+$ concentration increase and that MAPD decreases during myocardial ischemia (6). In the diltiazem-treatment group, onset of increase in [K$^+$], was much delayed and changes in [K$^+$], during the 3-min occlusion were also significantly less than those in the control group (Table 2). Diltiazem also significantly inhibited the shortening of MAPD and the decrease in pH$_e$ during ischemia (2). Therefore, this compound is likely to inhibit the following ionic and electrophysiological changes during ischemia: i) opening of K$_{ATP}$ channels which increases [K$^+$]; and shortens MAPD.

### Table 1. Time course of changes in hemodynamic variables and thickening fraction during ischemia and reperfusion

| Groups      | BP (mmHg) | HR (beats/min) | LVP (mmHg) | dP/dt$_{max}$ (mmHg/s) | TF (%) | CBF (ml/min) |
|-------------|-----------|----------------|-------------|------------------------|--------|--------------|
| Control     |           |                |             |                        |        |              |
| baseline    | 113 ± 3   | 160 ± 8        | 115 ± 6     | 2104 ± 41              | 15.9 ± 2.6 | 21 ± 0.6    |
| 8-min occlusion | 108 ± 5   | 164 ± 10       | 114 ± 9     | 1924 ± 140             | −5.5 ± 3.5$^*$ | 0          |
| 10-min reperfusion | 109 ± 4   | 164 ± 10       | 118 ± 9     | 1812 ± 67              | 3.1 ± 2.7 | 20 ± 1.7    |
| 120-min reperfusion | 111 ± 5   | 177 ± 12       | 121 ± 12    | 1890 ± 176             | 0.7 ± 2.1$^*$ | 20 ± 0.4    |
| Diltiazem   |           |                |             |                        |        |              |
| baseline    | 95 ± 9    | 148 ± 13       | 110 ± 11    | 1840 ± 308             | 11.2 ± 1.9 | 21 ± 1.2    |
| 8 min after administration | 87 ± 7    | 147 ± 13       | 107 ± 11    | 1820 ± 329             | 10.8 ± 2.1 | 26 ± 4.0    |
| 8-min occlusion | 86 ± 6$^*$ | 133 ± 10       | 109 ± 11    | 1472 ± 330             | −8.9 ± 3.7$^*$ | 0          |
| 10-min reperfusion | 85 ± 6$^*$ | 126 ± 12       | 111 ± 12    | 1600 ± 281             | 10.1 ± 4.2 | 26 ± 5.3    |
| 120-min reperfusion | 95 ± 8    | 143 ± 17       | 121 ± 18    | 1860 ± 331             | 11.8 ± 2.8$^*$ | 23 ± 2.6    |

BP indicates mean arterial pressure; HR, heart rate; LVP, left ventricular pressure; dP/dt$_{max}$, maximum of the first derivation of LVP; TF, thickening fraction; CBF, coronary blood flow. Values are means ± S.E.M. (n = 5). *P<0.05, **P<0.01: vs control group. $^*$P<0.05, $^*$P<0.01 vs baseline value.
Table 2. Time course of changes in monophasic action potential duration, extracellular potassium and pH during ischemia

| Groups            | MAPD (ms) | [K+]e (mM) | pH  |
|-------------------|-----------|------------|-----|
| Control           |           |            |     |
| baseline value    | 138 ± 8   | 3.28 ± 0.13| 7.36 ± 0.03|
| 1-min occlusion   | 103 ± 12* | 4.65 ± 0.57| 7.35 ± 0.03|
| 3-min occlusion   | 93 ± 14** | 7.85 ± 1.24*| 7.22 ± 0.03|
| 8-min occlusion   | 109 ± 5   | 8.83 ± 1.51**| 7.06 ± 0.05**|
| 10-min reperfusion| 129 ± 12  | 3.93 ± 0.37| 7.44 ± 0.02|
| 120-min reperfusion| 148 ± 8  | 4.39 ± 0.70| 7.36 ± 0.06|
| Diltiazem         |           |            |     |
| baseline value    | 140 ± 8   | 3.66 ± 0.09| 7.42 ± 0.04|
| 8 min after ad  | 143 ± 7** | 3.75 ± 0.20| 7.39 ± 0.05|
| 1-min occlusion   | 141 ± 9   | 3.75 ± 0.32| 7.40 ± 0.05|
| 3-min occlusion   | 120 ± 5   | 4.83 ± 0.51*| 7.34 ± 0.04*|
| 8-min occlusion   | 121 ± 11  | 7.34 ± 1.41**| 7.17 ± 0.06**|
| 10-min reperfusion| 144 ± 7   | 3.53 ± 0.26| 7.32 ± 0.06|
| 120-min reperfusion| 151 ± 9  | 3.53 ± 0.17| 7.34 ± 0.09|

MAPD indicates monophasic action potential duration at 60% repolarization; [K+]e, extracellular potassium concentration; pHx, extracellular pH. Values are means ± S.E.M. (n = 5). *P < 0.05, **P < 0.01: vs control group. ***P < 0.001: vs baseline value.

The present finding that diltiazem did not cause the shortening of MAPD, although it possesses a potent myocardial protection effect, strengthens our previous results obtained when testing pinacidil and preconditioning (7). We have shown that ischemic preconditioning improves contractile function, while it abolishes shortening of MAPD and blunts the rise of [K+]e during ischemia (7). In contrast, pinacidil which also produces myocardial protection, did not alter the shortening of MAPD and [K+]e elevation during ischemia (7). These overall findings suggest that shortening of MAPD is not a prerequisite for amelioration of contractile dysfunction by a KATP channel opener or ischemic preconditioning. Moreover, the results of present study also suggest that amelioration of myocardial contractile dysfunction by diltiazem is not mediated by the shortening of action potential duration.

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