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EFFECTS OF MCI-176, A NEW QUINAZOLINONE CALCIUM ANTAGONIST, ON MYOCARDIAL ENERGY AND CARBOHYDRATE METABOLISM IN ISCHEMIC DOG HEARTS

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Abstract—The effect of 2-(2,5-dimethoxyphenylmethyl)-3-(2-dimethylaminoethyl)-6-isopropoxy-4(3*H*)-quinazolinone hydrochloride (MCI-176), a calcium antagonist, on ischemic myocardial metabolism was studied in dog hearts subjected to an occlusion of the left anterior descending coronary artery (LAD) for 3 or 30 min. MCI-176 (0.03 or 0.1 mg/kg), when injected i.v. 5 min before occlusion, increased coronary blood flow and decreased systemic aortic pressure. When the LAD was ligated, the levels of creatine phosphate, ATP, total adenine nucleotides and energy charge potential decreased in the ischemic myocardium. Three minutes after ischemia, MCI-176 (0.1 mg/kg) significantly ($P < 0.05$) diminished these impairments of energy metabolism. Even 30 min after ischemia, pretreatment with MCI-176 tended to lessen the depletion of ATP and total adenine nucleotides, although these effects were not statistically significant. Myocardial ischemia produced a breakdown of glycogen, an accumulation of lactate, and an inhibition of glycolytic flux through phosphofructokinase reaction. MCI-176 (0.1 mg/kg) significantly ($P < 0.05$) reduced these alterations of carbohydrate metabolism after 3 min of ischemia. These results suggest that pretreatment with MCI-176 reduces the impairments of myocardial energy and carbohydrate metabolism in ischemic dog hearts, suggesting that the drug is capable of improving the imbalance between oxygen supply and oxygen demand in the ischemic myocardium.

There are a number of reports suggesting that calcium antagonists protect the myocardium from ischemic damage [1–3]. The anti-ischemic action of calcium antagonists has been considered to improve the imbalance between oxygen supply and oxygen demand of the heart through coronary and systemic vasodilation. Furthermore, beneficial effects of the drugs on ischemic myocardium are also associated with reduced high energy phosphate catabolism [4]. In our previous studies, however, diltiazem, a benzothiazepine calcium antagonist, reduced the depletion of myocardial high energy phosphates due to coronary artery ligation in dogs [5], whereas nifedipine and niludipine, dihydropyridine calcium antagonists, had no such beneficial effects on ischemic myocardial metabolism [6, 7]. Dihydropyridine calcium antagonists have been demonstrated to be more vasoselective than the other calcium antagonists [8]. This selectivity may result in the conflicting effects of calcium antagonists on ischemic myocardial metabolism, but precise mechanisms are still unclear. Therefore, it is important to elucidate the effect of a new type of calcium antagonist on myocardial metabolism for understanding the pathophysiology of myocardial ischemia.

2-(2,5-Dimethoxyphenylmethyl)-3-(2-dimethylaminoethyl)-6-isopropoxy-4 (3*H*)-quinazolinone hydrochloride (MCI-176) is a newly synthesized calcium antagonist (Fig. 1), which is one of the quinazolinone derivatives and is structurally unrelated to representative drugs such as nifedipine, diltiazem and verapamil [9–12]. MCI-176 has been shown to produce high selective vasodilation to large coronary arteries in isolated porcine coronary arteries [10] and in anesthetized open chest dogs [13], suggesting that the drug is capable of improving regional perfusion to the ischemic area. Furthermore, Hosono and Taira [11] have reported that negative inotropic action of MCI-176 is far less potent than that of the other calcium antagonists, suggesting that the drug is unlikely to produce cardiac failure. In the present study, therefore, the effects of MCI-176 on myocardial energy and carbohydrate metabolism were examined in ischemic dog hearts.

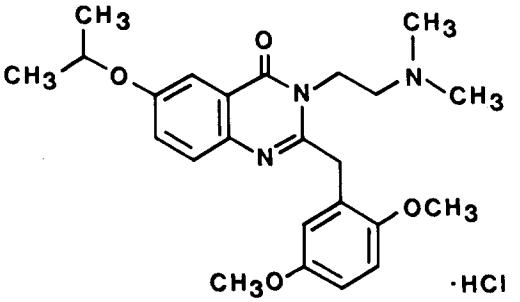

Fig. 1. Chemical structure of MCI-176.

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MATERIALS AND METHODS

Animal preparation. Seventy-two healthy mongrel dogs of either sex weighing 7–29 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). Dogs were ventilated with a positive-pressure respirator (Harvard Apparatus Co., Inc., Mills, MA, U.S.A.) using room air. A left thoracotomy was performed at the fourth intercostal space and the left ventricular wall was exposed. A main trunk of the left anterior descending coronary artery (LAD)‡ was dissected free from the adjacent tissues at a distal portion to the first diagonal branch and was loosely encircled with a 2-0 silk thread ligature. Coronary blood flow was measured with an electromagnetic flow probe (FR-020T, Nihon Kohden, Tokyo, Japan) placed around the LAD at just proximal to the ligature. Systemic aortic pressure was measured with a pressure transducer (MPU-0.5, Nihon Kohden) in the left carotid artery. Heart rate was counted from ECG taken in the standard limb lead II.

Experimental protocol. After a 60-min period of stabilization, either saline solution or MCI-176 solution (0.03 or 0.1 mg/kg) was injected i.v. over a period of 30 sec. Five minutes after the drug injection, a main trunk of LAD was ligated by a silk thread. A full thickness sample of the myocardium was removed rapidly with scissors from the center of the ischemic area either 3 min or 30 min after the ligation of LAD. An ischemic region of the myocardium was assessed by visible cyanosis and the elevation of the ST segment of epicardial ECG which was taken by a wire electrode attached on the left ventricular wall. The myocardium was also removed from the LAD area without ligating LAD 5 min after the drug injection. The samples were immediately pressed and frozen with clamps previously chilled in liquid nitrogen in such a way that the endocardial portion of the myocardium could be taken separately for analysis [14].

Biochemical analysis. The endocardial tissue sample was pulverized in a mortar with a pestle precooled with liquid nitrogen and extracted with perchloric acid. The levels of glycogen, glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), fructose-1,6-diphosphate (FDP), lactate, ATP, ADP, AMP and creatine phosphate (CrP) in neutralized perchloric acid extract were determined according to standard enzymatic procedures [15].

Drugs. MCI-176 was dissolved in saline solution immediately before use. The volume of a bolus injection of saline or MCI-176 solution was 0.5 mL/kg.

Calculation. Energy charge potential (ECP) was calculated from the levels of ATP, ADP and AMP to estimate the myocardial energy state, according to the equation $([ATP] + 1/2[ADP])/([ATP] + [ADP] + [AMP])$ [16]. The ratio of $([G6P] + [F6P])/[FDP]$ was calculated from the concentration of hexose phosphates to assess the rate of glycolytic flux through phosphofructokinase (PFK) reaction [2, 5, 17]. Hemodynamic data were evaluated by a paired Student's *t*-test, and biochemical data were analyzed by one-way analysis of variance followed by Dunnett's *t*-test. P values less than 0.05 were considered significant.

‡ Abbreviations: LAD, left anterior descending coronary artery; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FDP, fructose,1,6-diphosphate; CrP, creatine phosphate; ECP, energy charge potential; and PFK, phosphofructokinase.

RESULTS

Systemic hemodynamics. Hemodynamic data from the dogs whose LAD had been ligated for 30 min are summarized in Table 1. Saline administration had no significant effect on systemic blood pressure or coronary blood flow, and at both 3- and 30-min post-LAD ligation, the saline control treated hearts manifested significant reductions in blood pressure without reflex tachycardia, and showed significant elevation of the ST segment of epicardial ECG. A bolus injection of MCI-176 (0.03 or 0.1 mg/kg) decreased systemic blood pressure, increased heart rate slightly, probably because of reflex tachycardia, and increased coronary blood flow. In dogs pretreated with either dose of MCI-176, LAD ligation resulted in decreases in blood pressure and ST segment elevation of epicardial ECG, similar to those in saline-treated dogs. Comparable hemodynamic changes were observed in dogs in MCI-pretreated ligated coronary and non-ligated hearts for 3 min.

Energy metabolism. Changes in subendocardial CrP levels in both ischemic and non-ischemic myocardium are shown in Table 2. Myocardial CrP concentrations were reduced significantly at 3- and 30-min post-LAD ligation in saline- and MCI-176-treated hearts. Pretreatment with MCI-176, however, attenuated the depletion of CrP during ischemia. But only in high dose MCI-176 (0.1 mg/kg) treated hearts were CrP levels significantly higher at 3-min post-LAD ligation, as compared to saline-treated controls.

Changes in adenine nucleotide concentrations during ischemia are shown in Table 3. Three minutes post-LAD ligation, ATP levels decreased significantly while ADP and AMP levels increased. Ischemic zone myocardial ATP was further depleted 30 min post-ligation. In contrast to the rapid CrP depletion, the decrease in ischemic zone ATP levels evolved more slowly. Total adenine nucleotide levels also decreased in a time-related manner. MCI-176-treated hearts attenuated the depletion of both ATP and total adenine nucleotides, as contrasted with the saline control; but only high dose MCI-176 (0.1 mg/kg) treated hearts at 3-min post-LAD ligation were these changes statistically significant compared to saline controls.

To estimate the myocardial energy state, we calculated ECP from the levels of adenine nucleotides. As shown in Fig. 2, ECP decreased as a function of the ischemic period, and pretreatment with MCI-176 lessened the decrease in ECP caused by ischemia; 3 min after ischemia, ECP in the MCI-176 (0.1 mg/kg) treated heart was significantly higher than that in saline treated heart.

Carbohydrate metabolism. The levels of glycogen in non-ischemic and ischemic myocardium are shown in Table 4. The tissue level of glycogen decreased during ischemia. Pretreatment with MCI-176

Table 1. Changes in heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), coronary blood flow (CBF) and ST segment of epicardial ECG (ST) in saline- and MCI-176-treated dogs whose LAD was ligated for 30 min

	HR (beats/min)	SBP (mm Hg)	DBP (mm Hg)	CBF (mL/min)	ST (mV)
Saline-treated (N = 9)					
Before injection	148.7 ± 6.6	115.0 ± 8.6	83.0 ± 7.3	15.8 ± 3.9	
Maximum response	—	—	—	—	
5 min after injection	148.6 ± 6.6	115.2 ± 8.2	84.3 ± 7.4	15.8 ± 3.9	
3 min after ligation	149.8 ± 6.6	107.7 ± 8.1*†	77.3 ± 6.4*†		11.2 ± 0.7*†
30 min after ligation	149.0 ± 6.5	105.4 ± 7.4*†	79.4 ± 7.5*†		11.2 ± 1.4*†
MCI-176-treated (0.03 mg/kg) (N = 7)					
Before injection	139.3 ± 8.1	133.1 ± 10.1	101.7 ± 7.5	11.5 ± 1.0	
Maximum response	142.9 ± 9.0	116.1 ± 7.7*	84.3 ± 5.4*	18.5 ± 0.5*	
5 min after injection	137.7 ± 8.0	130.3 ± 9.9	98.7 ± 8.1*	11.8 ± 1.2	
3 min after ligation	139.4 ± 8.2	123.0 ± 8.8*†	93.7 ± 7.2†		14.6 ± 2.0*†
30 min after ligation	140.6 ± 8.4	126.6 ± 9.9	97.4 ± 8.5		14.0 ± 2.3*†
MCI-176-treated (0.1 mg/kg) (N = 8)					
Before injection	142.6 ± 6.6	115.6 ± 5.6	87.0 ± 5.5	11.8 ± 0.8	
Maximum response	147.4 ± 6.4*	89.6 ± 4.9*	56.0 ± 4.8*	20.8 ± 1.0*	
5 min after injection	139.1 ± 6.7*	110.1 ± 4.5*	80.9 ± 4.1*	13.2 ± 1.0	
3 min after ligation	137.4 ± 6.9	99.9 ± 4.7*†	73.6 ± 3.6*†		13.1 ± 1.1*†
30 min after ligation	140.0 ± 6.4	102.1 ± 5.1*	78.1 ± 4.0		13.3 ± 1.6*†

Saline or MCI-176 (0.03 or 0.1 mg/kg) was injected i.v. Five minutes after the injection, a main trunk of LAD was ligated for 30 min. Values are means ± SEM. The numbers of dogs (N) are shown in parentheses.

* $P < 0.05$, compared with "before injection" in each group.

† $P < 0.05$, compared with "5 min after injection" in each group.

Table 2. Changes in creatine phosphate level during ischemia in saline- and MCI-176-treated dogs

	N	CrP (μmol/g wet tissue)
Saline-treated		
Non-ischemic myocardium	9	6.218 ± 0.407
Ischemic myocardium, 3 min	8	1.484 ± 0.158*
Ischemic myocardium, 30 min	9	1.967 ± 0.386*
MCI-176-treated (0.03 mg/kg)		
Non-ischemic myocardium	7	6.219 ± 0.472
Ischemic myocardium, 3 min	8	2.493 ± 0.462*
Ischemic myocardium, 30 min	7	1.678 ± 0.234*
MCI-176-treated (0.1 mg/kg)		
Non-ischemic myocardium	8	5.846 ± 0.450
Ischemic myocardium, 3 min	8	2.993 ± 0.454*†
Ischemic myocardium, 30 min	8	2.386 ± 0.685*

The myocardial samples, whose LAD was not ligated (non-ischemic myocardium) or ligated for 3 min (ischemic myocardium, 3 min) or ligated for 30 min (ischemic myocardium, 30 min) in saline- and MCI-176-treated dogs, were removed from the center of the ischemic area and immediately frozen in liquid nitrogen. Values are means ± SEM. N = the number of dogs in each group.

* $P < 0.05$, compared with "non-ischemic myocardium" in each group.

† $P < 0.05$, compared with the corresponding value of the "saline-treated" group.

Table 3. Changes in levels of adenine nucleotides during ischemia in saline- and MCI-176-treated dogs

	N	ATP	ADP	AMP	Total
		(μmol/g wet tissue)			
Saline-treated					
Non-ischemic myocardium	9	4.970 ± 0.098	0.931 ± 0.029	0.176 ± 0.010	6.077 ± 0.101
Ischemic myocardium, 3 min	8	3.893 ± 0.089*	1.346 ± 0.040*	0.295 ± 0.018*	5.534 ± 0.088*
Ischemic myocardium, 30 min	9	1.751 ± 0.167*	0.840 ± 0.032	0.363 ± 0.076*	2.958 ± 0.102*
MCI-176-treated (0.03 mg/kg)					
Non-ischemic myocardium	7	5.032 ± 0.096	0.975 ± 0.025	0.192 ± 0.012	6.185 ± 0.104
Ischemic myocardium, 3 min	8	4.277 ± 0.151*	1.395 ± 0.099*	0.231 ± 0.024	5.903 ± 0.136
Ischemic myocardium, 30 min	7	1.984 ± 0.112*	0.934 ± 0.028	0.285 ± 0.060	3.204 ± 0.103*
MCI-176-treated (0.1 mg/kg)					
Non-ischemic myocardium	8	5.068 ± 140	0.987 ± 0.070	0.202 ± 0.014	6.258 ± 0.197
Ischemic myocardium, 3 min	8	4.321 ± 0.107†	1.200 ± 0.061*	0.235 ± 0.018	5.756 ± 0.063*
Ischemic myocardium, 30 min	8	2.201 ± 0.310*	0.940 ± 0.045	0.374 ± 0.077*	3.516 ± 0.224*

Values are means ± SEM. See Table 2 for experimental details.

* $P < 0.05$, compared with "non-ischemic myocardium" in each group.

† $P < 0.05$, compared with the corresponding value of the "saline-treated" group.

(0.1 mg/kg) significantly lessened the decrease in the glycogen level caused by 3 min of ischemia.

Changes in the levels of hexose phosphates during ischemia are summarized in Table 5. We determined the levels of G6P, F6P and FDP in the myocardium with or without coronary ligation of LAD, and calculated the ratio of ([G6P] + [F6P])/[FDP] to assess the rate of glycolytic flux through PFK reaction. The levels of G6P and F6P increased in the ischemic myocardium, whereas the level of FDP decreased, and therefore the ratio of ([G6P] + [F6P])/[FDP] increased over ten times during ischemia. MCI-176 significantly prevented the decrease in the level of FDP and the increase in the ratio of ([G6P] + [F6P])/[FDP] after 3 min of ischemia (Fig. 3).

The level of lactate increased significantly after ischemia, depening on the period of ischemia. Three minutes after ischemia the increase in the level of lactate was reduced significantly by pretreatment with MCI-176 at a dose of 0.1 mg/kg (Table 6).

DISCUSSION

Myocardial ischemia occurring secondary to coronary occlusion results in depletion of high energy phosphates with a switch from aerobic to anaerobic

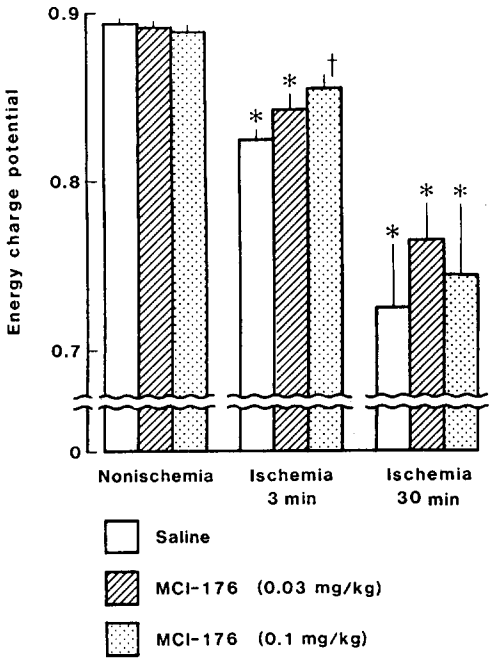

Fig. 2. Effect of MCI-176 on changes in ECP during ischemia. ECP was calculated from the levels of adenine nucleotides in the myocardium, whose LAD was not ligated ("Nonischemia", left) or ligated for 3 min ("Ischemia 3 min", middle) or ligated for 30 min ("Ischemia 30 min", right), according to the equation ([ATP] + 1/2[ADP])/([ATP] + [ADP] + [AMP]). Saline (□), MCI-176 (0.03 mg/kg) (▨) or MCI-176 (0.1 mg/kg) (▩) was injected 5 min before ligation. Data are means ± SEM of 7–9 observations in each group. Key: (*) $P < 0.05$, compared with "Nonischemia" in each group; and (†) $P < 0.05$, compared with the corresponding value of the "Saline" group.

metabolism. During the anaerobic state, the rate of glycolytic flux could be accelerated to produce ATP anaerobically. In the present study, however, glycolysis was not stimulated during ischemia, in which the myocardium must be in anaerobic conditions. The negative crossover point between F6P and FDP was obtained in the ischemic myocardium (Table 5), suggesting an inhibition of glycolytic flux through PFK reaction. Without a direct measurement of glycolytic flux, static measurements of the substrates and products of a reaction do not quantify the rate of glycolytic flux. Opie [18] has stated clearly in his paper that measurement of glycolytic flux in regionally ischemic myocardium is not easy. Why was anaerobic glycolysis inhibited at the level of PKF during ischemia? Myocardial glycogen is consumed and lactate accumulates, resulting in tissue acidosis [19, 20]. Hydrogen ions inhibit PKF reaction [21, 22].

In the present study, a quinazolinone calcium antagonist, MCI-176, attenuated the depletion of high energy phosphate stores and delayed the switching from aerobic to anaerobic metabolism in ischemic myocardium. MCI-176 also partially reversed the ischemic related inhibition of glycolytic flux, as calculated from the ratio of ([G6P] + [F6P])/[FDP], possibly by increasing myocardial pH [23]. MCI-176 may, therefore, partially reverse an ischemia-induced inhibition of anaerobic glycolysis, as mediated by the PFK reaction.

In our previous studies, diltiazem attentuated myocardial acidosis and the increase in the ratio of ([G6P] + [F6P])/[FDP], whereas nifedipine and niludipine did not [5–7, 24]. It is likely, therefore, that not all calcium antagonists are similar. They vary in vasoselectivity versus cardioselectivity which has important implications for coronary vasodilation and potentially negative inotropic effects, both of which affect myocardial perfusion [25]. Dihydropyridine calcium antagonists, as compared to non-dihydropyridine calcium antagonists and quinazolinone calcium antagonists, may have deleterious

Table 4. Changes in glycogen level during ischemia in saline- and MCI-176-treated dogs

	N	Glycogen (μmol glucose equivalent/g wet tissue)
Saline-treated		
Non-ischemic myocardium	9	28.319 ± 1.738
Ischemic myocardium, 3 min	8	17.253 ± 2.251*
Ischemic myocardium, 30 min	9	9.671 ± 1.391*
MCI-176-treated (0.03 mg/kg)		
Non-ischemic myocardium	7	31.889 ± 1.373
Ischemic myocardium, 3 min	8	18.831 ± 2.509*
Ischemic myocardium, 30 min	7	13.340 ± 2.076*
MCI-176-treated (0.1 mg/kg)		
Non-ischemic myocardium	8	29.359 ± 3.796
Ischemic myocardium, 3 min	8	26.276 ± 2.415†
Ischemic myocardium, 30 min	8	8.989 ± 1.956*

Values are means ± SEM. See Table 2 for experimental details.
* $P < 0.05$, compared with "non-ischemic myocardium" in each group.
† $P < 0.05$, compared with the corresponding value of the "saline-treated" group.

Table 5. Changes in the levels of hexose phosphates during ischemia in saline- and MCI-176-treated dogs

	N	G6P	F6P (μmol/g wet tissue)	FDP
Saline-treated				
Non-ischemic myocardium	9	0.217 ± 0.040	0.038 ± 0.006	0.117 ± 0.012
Ischemic myocardium, 3 min	8	1.392 ± 0.162*	0.345 ± 0.053*	0.054 ± 0.004*
Ischemic myocardium, 30 min	9	1.184 ± 0.167*	0.338 ± 0.048*	0.062 ± 0.007*
MCI-176-treated (0.03 mg/kg)				
Non-ischemic myocardium	7	0.188 ± 0.026	0.042 ± 0.004	0.101 ± 0.017
Ischemic myocardium, 3 min	8	1.006 ± 0.243*	0.218 ± 0.072*	0.113 ± 0.012†
Ischemic myocardium, 30 min	7	1.564 ± 0.355*	0.431 ± 0.099*	0.076 ± 0.012
MCI-176-treated (0.1 mg/kg)				
Non-ischemic myocardium	8	0.207 ± 0.019	0.045 ± 0.011	0.079 ± 0.013
Ischemic myocardium, 3 min	8	0.814 ± 0.134*	0.195 ± 0.037*	0.084 ± 0.006†
Ischemic myocardium, 30 min	8	1.106 ± 0.170*	0.301 ± 0.048*	0.074 ± 0.011

Values are means ± SEM. See Table 2 for experimental details.
* $P < 0.05$, compared with "non-ischemic myocardium" in each group.
† $P < 0.05$, compared with the corresponding value of the "saline-treated" group.

Table 6. Changes in lactate level during ischemia in saline- and MCI-176-treated dogs

	N	Lactate (μmol/g wet tissue)
Saline-treated		
Non-ischemic myocardium	9	1.188 ± 0.403
Ischemic myocardium, 3 min	8	10.290 ± 0.490*
Ischemic myocardium, 30 min	9	26.961 ± 2.559*
MCI-176-treated (0.03 mg/kg)		
Non-ischemic myocardium	7	1.363 ± 0.272
Ishcemic myocardium, 3 min	8	8.631 ± 1.130*
Ischemic myocardium, 30 min	7	25.988 ± 2.415*
MCI-176-treated (0.1 mg/kg)		
Non-ischemic myocardium	8	1.624 ± 0.469
Ischemic myocardium, 3 min	8	6.522 ± 0.774†
Ischemic myocardium, 30 min	8	24.390 ± 3.414*

Values are means ± SEM. See Table 2 for experimental details.
* $P < 0.05$, compared with "non-ischemic myocardium" in each group.
† $P < 0.05$, compared with the corresponding value of the "saline-treated" group.

effects on ischemic myocardial blood flow and metabolism, particularly if coronary stenosis is severe and autoregulatory coronary flow reserve is exhausted. In fact, nifedipine has been demonstrated to decrease ischemic myocardial blood flow and to cause a redistribution of myocardial blood flow from endocardium to epicardium [26–28]. The potent coronary vasodilator effect of dihydropyridine calcium antagonists, therefore, may cause a "coronary steal" of blood flow away from ischemic zone subendocardium. Moreover, a dilatation of large conductive coronary arteries, rather than that of small resistive arterioles, improves the regional perfusion to the ischemic area [29]. In this context, it is of interest that not nifedipine, but MCI-176 and diltiazem dilate large coronary arteries more selectively in isolated porcine coronary arteries [10] and in anesthetized open chest dogs [13]. Accordingly, the favorable effects of MCI-176 and diltiazem on the ischemic myocardium could be due to its vasolidating effect on the large coronary arteries.

In conclusion, pretreatment with MCI-176 reduced the impairments of myocardial energy and carbohydrate metabolism in ischemic dog hearts, suggesting that MCI-176 could be capable of improving the imbalance between oxygen supply and oxygen demand in the ischemic myocardium.

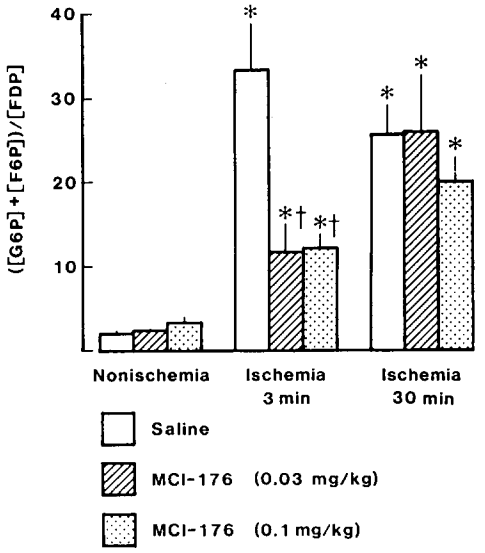

Fig. 3. Effect of MCI-176 on the ratio of ([G6P] + [F6P])/[FDP] in the ischemic myocardium. The ratio of ([G6P] + [F6P])/[FDP] was calculated from the levels of hexose phosphates. Symbols are the same as those described in Fig. 2. Data are means ± SEM of 7–9 observations in each group. Key: (*) $P < 0.05$, compared with "Nonischemia" in each group; and (†) $P = < 0.05$, compared with the corresponding value of the "Saline" group.

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