ELSEVIER

European Journal of Pharmacology 323 (1997) 197–204

ejp

Y-26763 protects the canine heart from a stunning injury through opening of the K_{ATP} channels

Atsushi Fukunari, Hisato Miyai, Kenji Shinagawa, Kiminori Kawahara, Tohru Nakajima *

Research Laboratories, Yoshitomi Pharmaceutical Industries, Ltd., 955 Koiwai, Yoshitomi-cho, Chikujo-gun, Fukuoka 871, Japan

Received 2 January 1997; accepted 7 January 1997

Abstract

The present study describes the protective effects of the ATP-sensitive K^+ (K_{ATP}) channel opener Y-26763 ((−)-(3*S*,4*R*)-4-(*N*-acetyl-*N*-hydroxyamino)-6-cyano-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ol), on a model of reversible ischemia/reperfusion injury ('stunned' myocardium). Stunning was caused by 10-min occlusion of the left circumflex coronary artery followed by 3-h reperfusion in pentobarbital anesthetized beagle dogs. This procedure reduced by over 80% myocardial segment function measured by sonomicrometry in control preparations. Y-26763, administered 10 min before the left circumflex coronary artery occlusion, at a dose (3 μg/kg, i.v.) lacking significant systemic hemodynamic effects, produced a rapid and marked (80%) recovery of the shortening of the ischemic segment. By contrast, nifedipine (1 μg/kg plus 0.2 μg/kg per min, i.v.) did not ameliorate postischemic function. Glibenclamide, administered before Y-26763 at a dose (0.3 mg/kg, i.v.) that did not affect adversely hemodynamics and stunning injury negated the beneficial action of Y-26763. However, glibenclamide failed to do so when administered 2 min before starting reperfusion. The ischemia/reperfusion areas, which were measured by digital image analysis with NIH Image software, were similar among experimental groups. Overall, these results indicate that Y-26763 protects the canine myocardium from reversible ischemia/reperfusion injury, probably through activation of myocardial K_{ATP} channels which appear to be involved in affording protection during the ischemic insult and not at the reperfusion.

Keywords: Y-26763; Myocardium, stunned; Ischemia; Reperfusion; ATP-sensitive K^+ channel

1. Introduction

The ATP-sensitive K^+ (K_{ATP}) channel was identified in isolated cardiac myocytes by Noma (1983). The most recent exciting development in this field is the increasing evidence that this channel is involved in the cardioprotection afforded by ischemic preconditioning on K^+ channel openers (Yellon et al., 1993; Baxter and Yellon, 1994; Lawson, 1994). Thus the pharmacology and the physiology of this channel is of therapeutic interest since it may allow the identification of means for reducing the size of ischemic infarcts and for accelerating the recovery of myocardial segment function after brief periods of coronary flow impairment (Cook, 1988; Auchampach et al., 1991; Gross and Auchampach, 1992; Yao and Gross, 1994a).

Postischemic left ventricular myocardial dysfunction, termed stunning, is characterized by a persistent but ultimately reversible depression of contractile function (Braunwald and Kloner, 1982). The protective procedure of stunning by drugs may be of therapeutic importance in several clinical settings, such as percutaneous transluminal coronary angioplasty or cardiac transplantation (D'Alomzo et al., 1992).

Y-26763 ((−)-(3*S*,4*R*)-4-(*N*-acetyl-*N*-hydroxyamino)-6-cyano-3,4-dihydro-2,2-dimethyl-2*H*-1-benzopyran-3-ol) is the active metabolite and is formed by the liver cytochrome P-450 system from an inactive form, Y-27152 (Nakajima, 1992). Y-26763 has been demonstrated to exert its pharmacological effects by opening K_{ATP} channels, and there is no major difference between Y-26763 and other potassium channel openers in in vitro and in vivo tests. Indeed, the direct vasorelaxant action of Y-26763 was competitively antagonized by the K_{ATP} channel blocker glibenclamide in rat aortic rings, and the hypotensive action of Y-26763 was also antagonized by glibenclamide in SHR (Nakajima et al., 1992). Y-27152 lowers blood pressure in hypertensive animals, but this effect is not

* Corresponding author. Tel.: (81-979) 23-8983; Fax: (81-979) 24-2769.

0014-2999/97/$17.00
PII S0014-2999(97)00035-6

accompanied by a substantial increase in heart rate (Nakajima et al., 1992). At present, the prolonged duration of action and gradual onset of action of Y-27152 in vivo are desirable properties for a potential drug to treat cardiovascular disease. We previously reported that intraduodenal dosing of Y-27152 markedly reduces the infarct size produced in dogs by 60-min occlusion of the left circumflex coronary artery and 5-h reperfusion (Nakajima, 1992), and Y-26763 protects the working rat myocardium from ischemia/reperfusion injury in vitro (Rahman et al., 1996). The present study on a model of reversible ischemia/reperfusion injury was undertaken to add further dimensions to the cardioprotective potential of Y-26763.

2. Materials and methods

2.1. General surgical preparation

Male and female beagle dogs (*Canis familiaris*) (Seiwa Experimental Animals, Fukuoka, Japan) were housed individually in stainless-steel mesh-bottomed cages under regulated temperature (23 ± 3°C), humidity (55 ± 5%) and a 12-h light/dark cycle before initiation of the study. A standard certified commercial laboratory diet (TC-1, Maruha, Tokyo, Japan) was available to each dog once daily, and water was provided ad libitum.

The animals weighing 8.3–11.7 kg were anesthetized with sodium pentobarbital (25 mg/kg, i.v.), incubated, and ventilated with a respirator (Model 607, Harvard Apparatus, South Natick, MA, USA) delivering room air. Arterial blood pH and gasses were maintained within normal physiological range (pH, 7.3–7.5; pCO_2, 25–40 mmHg; pO_2, 65–100 mmHg) by adjusting the respiration rate and/or by short (1 min) intravenous infusions of sodium pentobarbital (0.5 mg/kg) whenever necessary. Body temperature was maintained at about 38°C with a servomechanically controlled heating pad.

A polyethylene catheter was inserted through the right femoral artery into the aorta and connected to a tip pressure transducer catheter (MPC-500, Millar Instruments, Houston, TX, USA) for measuring arterial blood pressure. Left ventricular pressure was also measured with a tip pressure transducer catheter (SPC-350, Millar Instruments) directly inserted into the left ventricle through the left carotid artery. The first derivative of left ventricular pressure (dP/dt) was obtained by electric differentiation of the left ventricular pulse pressure. Heart rate was determined with a tachometer (NEC San-ei, Tokyo, Japan) triggered by blood pressure pulses. Drugs were administered into a cannulated right femoral vein.

The chest was opened at the level of the fifth intercostal space. The lung was carefully retracted, and the heart suspended in a pericardial cradle. A 1.0–1.5 cm segment of the left circumflex coronary artery was isolated from the surrounding tissue. The left circumflex coronary artery blood flow was continuously measured with a 20-MHz pulsed Doppler flow probe (HDP 20S, Crystal Biotech, Hopkinton, MA, USA) which was placed around the vessel immediately distal to the occluder (Disposable Clamp, Kyowa Tokei, Tokyo, Japan) by taking care that no evident arterial branch was present between the flow probe and the occluder. The mean arterial blood pressure, heart rate, left ventricular pressure, and dP/dt were recorded at various time intervals throughout the experiments on a polygraph (NEC San-ei). The left circumflex coronary artery blood flow and myocardial segment length were measured with a pulsed Doppler flowmeter (VF-1, Crystal Biotech) and displayed on a paper recorder (NEC San-ei).

2.2. Measurement of myocardial segment function

Myocardial segment function was measured in the regions perfused by the left circumflex coronary artery with a nontraumatic single-crystal pulsed Doppler system (Hartley et al., 1983; Pitsillides and Longhurst, 1993). The technique uses a pulse-echo mode of operation common to most single-crystal ultrasonic devices, and it has been demonstrated that there is an excellent correlation between myocardial blood flow and the myocardial thickening fraction during ischemia in open chest dogs (Edwards et al., 1992). It operates by integrating the velocity of the various myocardial layers that pass back and forth through a range-gated sample volume located within the myocardium at a fixed distance from the epicardial surface (Hartley et al., 1983). A single piezoelectric crystal probe (DMT 40-10P, Crystal Biotech) was attached by a surgical adhesive agent (Aron Alfa A, Sankyo, Tokyo, Japan) at the myocardial surface in the region perfused by the left circumflex coronary artery, and fixed by sewing the cut on the pericardial cradle. The range-gate depth (RGD) was adjusted at 7–9 mm from the epicardium. The leads of the crystal were connected to an ultrasonic amplifier (DMM-10, Crystal Biotech), which transforms the crystal-transmitted sound pulse into an electrical signal proportional to the motion of myocardial tissue at a fixed RGD. End-diastolic thickness (EDT) was determined at the beginning of the rise phase of positive dP/dt (onset of isovolumic contraction), and end-systolic thickness (EST) was determined at peak negative dP/dt. The thickening fraction (TF) was calculated with the equation $TF = (EST - EDT)/RGD$. The percent segment-shortening (%SS) results are normalized as percentage of baseline values by using the following equation: $\%SS = 100 \times TF/TFp$, where TFp is the thickening fraction measured before any treatment. Each time point reported is the average of the measurements obtained during at least five connected beats.

2.3. Experimental protocol

The experimental design included a control measurement of hemodynamics and segment shortening of steady-

state conditions following a sufficient postoperative equilibration period. Subsequently, the left circumflex coronary artery was occluded for 15 min and then reperfused for 3 h. All parameters were recorded continuously. Dogs were randomly assigned to one of the following six experimental groups: (a) control group; (b) Y-26763 (3 μg/kg, i.v.) injected as a bolus 10 min before occluding the left circumflex coronary artery; (c) nifedipine (1 μg/kg, i.v. bolus plus 0.2 μg/kg per min) starting from 10 min before occlusion of the left circumflex coronary artery until the reperfusion; (d) glibenclamide (0.3 mg/kg, i.v.) given as a bolus 20 min before occlusion; (e) Y-26763 given 10 min after pretreatment with glibenclamide; (f) glibenclamide (0.3 mg/kg, i.v.) administrated 2 min before starting reperfusion in dogs treated as in group (b). The doses of Y-26763 and nifedipine were selected in a way that they produced an increase in coronary blood flow of less than 30% with no or minor hypotensive effect. The control group received the vehicle of glibenclamide (10% dimethylformamide and 0.1 M NaOH), Y-26763 or nifedipine (0.1% dimethylformamide in physiological saline). The left circumflex coronary artery was occluded with an occluder for 15 min followed by a 3-h period of reperfusion. Because of the difference in duration of action, the drug was bolus injected 10 min before the left circumflex coronary artery occlusion for Y-26763 or bolus injected followed by infusion for nifedipine, respectively.

2.4. Determination of area-at-risk and infarction in the ischemic zone

After a 3-h reperfusion, the animals were deeply anesthetized with sodium pentobarbital (10 mg/kg, i.v.). The left circumflex coronary artery was reoccluded and cannulated just distal to the occlusion site. Then, 50 ml of saline and 50 ml of 1.5% Evans blue dye in saline were injected into the left circumflex coronary artery and left atrium, respectively. The left circumflex coronary artery perfusion territory was unstained whereas the remainder of the heart was stained blue. The animals were excited by intravenous injection of saturated potassium chloride solution. The heart was immediately removed, cut into a transverse section of the left ventricle (5–7 mm in width) which included myocardial surface attaching the single piezoelectric crystal probe. The sections were incubated at 37°C for 10–20 min in 1% 2,3,5-triphenyltetrazolium chloride (TTC) in 0.1 mol/l phosphate buffer (pH 7.4). TTC stained the noninfarcted myocardium brick-red, whereas the infarcted (irreversibly damaged) myocardium remained unstained. The color photographs of these sections were digitized with a flatbed image scanner (HP Scan Jet IICX, Hewlett-Packard) at a resolution of 288 dots per inch in black-and-white-photo mode. The analyses of the entire area of left ventricle and the area-at-risk were performed on a Macintosh Quadra 840AV computer with a public domain NIH Image program. NIH Image was written by Wayne Rasband at the US National Institutes of Health, and is available from the Internet by anonymous ftp from zippy.nimh.nih.bov or on floppy disks from NTIS, 5285 Port Royal Road, Springfield, VA 22161, USA, part number PB93-504868.

2.5. Exclusion criteria

Animal exclusion from data analysis was based on the following criteria: (1) ventricular fibrillation at any point throughout the experiment, (2) lack of area-at-risk, or presence of infarction (nonstained by TTC) in area-at-risk.

2.6. Chemicals

Y-26763 and nifedipine were synthesized by Yoshitomi Research Laboratories (Fukuoka, Japan). Glibenclamide and sodium pentobarbital were purchased from Sigma (St. Louis, MO, USA) and Abbott Laboratories (North Chicago, IL, USA), respectively.

Table 1
Hemodynamic data of vehicle- and drug-treated dogs

Period	MBP (mmHg)	HR (beats/min)	CBF (ml/min)
Pretreatment			
Control ($n = 5$)	119 ± 7	167 ± 9	15 ± 1
Y-26763 ($n = 5$)	123 ± 8	152 ± 7	18 ± 1
Nifedipine ($n = 4$)	111 ± 6	155 ± 10	15 ± 2
Drug treatment (8 min)			
Control	119 ± 6	169 ± 10	16 ± 2
Y-26763	107 ± 10 [a]	161 ± 5	22 ± 1
Nifedipine	100 ± 4	164 ± 10	19 ± 3
Occlusion (10 min)			
Control	111 ± 6	161 ± 9	0 ± 0
Y-26763	100 ± 8 [a]	161 ± 5	0 ± 0
Nifedipine	91 ± 5 [a]	155 ± 9	0 ± 0
Reperfusion (10 min)			
Control	113 ± 4	172 ± 10	27 ± 5 [a]
Y-26763	111 ± 9	160 ± 5	36 ± 6 [a]
Nifedipine	98 ± 3	155 ± 8	41 ± 8 [a]
Reperfusion (1 h)			
Control	118 ± 5	161 ± 7	15 ± 2
Y-26763	114 ± 7	161 ± 5	23 ± 3
Nifedipine	108 ± 6	148 ± 10	15 ± 1
Reperfusion (3 h)			
Control	124 ± 5	161 ± 9	15 ± 2
Y-26763	120 ± 3	162 ± 9	21 ± 2
Nifedipine	113 ± 7	155 ± 8	19 ± 2

Control, vehicle-treated group; MBP, mean arterial blood pressure; HR, heart rate; CBF, circumflex coronary artery blood flow. Values are mean ± S.E.M.

[a] $P < 0.05$ vs. pretreatment value (two-way ANOVA, Dunnett test).

2.7. Data analysis

All reported values were expressed as means ± S.E.M. Differences in hemodynamics, segment shortening and the area-at-risk among groups were compared by one-way analysis of variance (ANOVA) and Tukey's test. Differences in hemodynamic responses within individual groups were compared by two-way ANOVA for repeated measures and the Dunnett test. A $P < 0.05$ was the criterion for statistical significance.

3. Results

3.1. Mortality and exclusions

Forty-two dogs were initially entered into the present study. Seventeen dogs were excluded, due to ventricular fibrillation during ischemia or reperfusion. This occurred in 1–4 dogs per group. Furthermore, one dog each in the control group, the glibenclamide-alone group, and the glibenclamide pretreatment plus Y-26763 group was excluded due to the lack of area-at-risk in the left ventricle. Thus, 25 animals were subjected to data analysis: 5 for the control group, 5 for the Y-26763-treated group, 4 for the nifedipine-treated group, 3 for the glibenclamide-treated group, 4 for the group receiving glibenclamide pretreatment plus Y-26763, and 4 for the group receiving glibenclamide posttreatment plus Y-26763.

Table 2
Hemodynamic data of glibenclamide-treated dogs before and after Y-26763 treatment

Period	MBP (mmHg)	HR (beats/min)	CBF (ml/min)
Pretreatment			
Y-26763 ($n = 5$)	123 ± 8	152 ± 7	18 ± 1
GL + Y ($n = 4$)	130 ± 13	162 ± 8	22 ± 3
Y + GL ($n = 4$)	112 ± 5	141 ± 8	19 ± 3
Drug treatment (8 min)			
Y-26763	107 ± 10 [a]	161 ± 5	22 ± 1
GL + Y	124 ± 12	166 ± 6	31 ± 3
Y + GL	99 ± 9	152 ± 11	29 ± 4
Occlusion (10 min)			
Y-26763	100 ± 8 [a]	161 ± 5	0 ± 0
GL + Y	110 ± 9	163 ± 5	0 ± 0
Y + GL	85 ± 3 [a]	143 ± 5	0 ± 0
Reperfusion (10 min)			
Y-26763	111 ± 9	160 ± 5	36 ± 6 [a]
GL + Y	109 ± 10	161 ± 5	40 ± 4 [a]
Y + GL	94 ± 5 [a]	145 ± 9	32 ± 7 [a]
Reperfusion (1 h)			
Y-26763	114 ± 7	161 ± 5	23 ± 3
GL + Y	117 ± 9	168 ± 5	27 ± 6
Y + GL	98 ± 1	146 ± 14	18 ± 2
Reperfusion (3 h)			
Y-26763	120 ± 3	162 ± 9	21 ± 2
GL + Y	116 ± 6	173 ± 7	29 ± 3
Y + GL	110 ± 2	146 ± 14	18 ± 3

MBP, mean arterial blood pressure; HR, heart rate; CBF, circumflex coronary artery blood flow; GL + Y, pretreatment with glibenclamide plus Y-26763; Y + GL, Y-26763 plus posttreatment with glibenclamide. Values are mean ± S.E.M.
[a] $P < 0.05$ vs. pretreatment value (two-way ANOVA, Dunnett test).

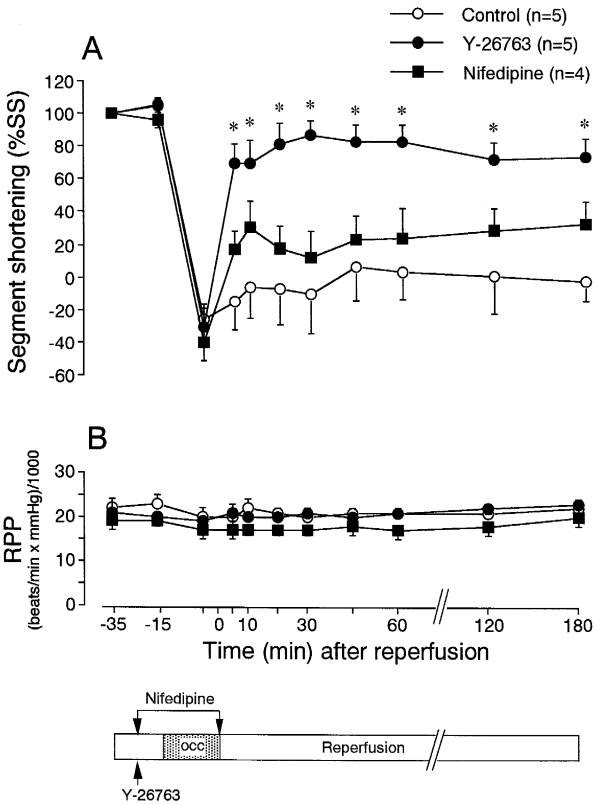

Fig. 1. Preservative effect of Y-26763 on the percentage of segment shortening (%SS) of the ischemic-reperfused area (A) and rate-pressure product (RPP, B). Y-26763 (3 μg/kg, i.v.) was administered as a bolus 10 min before the left circumflex coronary artery occlusion (OCC). Nifedipine was given as an intravenous dose of 1 μg/kg plus 0.2 μg/kg per min infusion beginning 10 min before the left circumflex coronary artery occlusion. Each point represents mean value. Bars indicate S.E.M. * $P < 0.05$ vs. control group (two-way ANOVA, Tukey's test).

3.2. Hemodynamics

Hemodynamic parameters for the dogs subjected to the left circumflex coronary artery occlusion and reperfusion are shown in Tables 1 and 2, and Figs. 1 and 2. In vehicle-treated (control) animals, hemodynamic parameters remained stable throughout the experimental procedure, except for the rate–left ventricular systolic pressure product which decreased slightly during the left circumflex coronary artery occlusion and early reperfusion periods. Y-26763, as compared to the vehicle control group, produced minor effects on the left circumflex coronary artery blood flow, mean arterial blood pressure, and rate–left

ventricular systolic pressure product before and after their administrations during the left circumflex coronary artery occlusion and at the reperfusion. Effects of nifedipine, pre-, and post-treatment of glibenclamide plus Y-26763 on the left circumflex coronary artery blood flow, mean blood pressure, and rate–pressure product were similar to that of Y-26763. Glibenclamide (0.3 mg/kg, i.v.) alone had no effect on these hemodynamic parameters (data not shown).

3.3. Myocardial segment shortening

The effects of Y-26763 and nifedipine on the recovery of postischemic function are shown in Fig. 1. In both drugs and vehicle-treated animals, %SS fell to negative values during the left circumflex coronary artery occlusion period, reflecting systolic bulging (myocardial stunning). In the vehicle-control group, less than 20% of the segment function in the ischemic region were recovered throughout the reperfusion period. Nifedipine, which was given at 1 μg/kg i.v. bolus 10 min before the occlusion, followed by a 0.2 μg/kg per min infusion which continued until the initiation of reperfusion, had no effect on this contractile dysfunction. By contrast, Y-26763 significantly improved the recovery of %SS, approximately 70% of the baseline value 5 min after starting reperfusion. Such an effect persisted throughout the 3-h reperfusion periods.

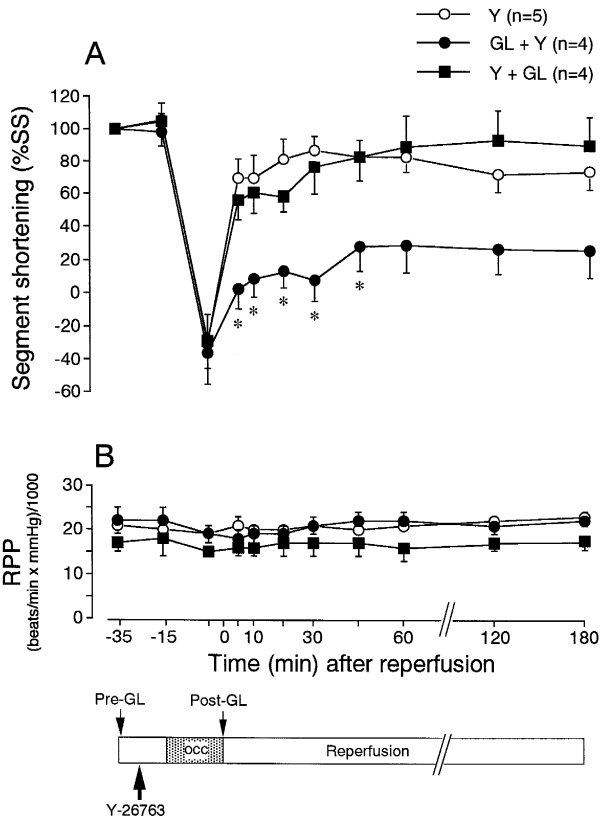

Fig. 2. Percentage of segment shortening (%SS) of the ischemic-reperfused area (A) and rate-pressure product (RPP, B). Y-26763 (3 μg/kg, i.v.) was administered as a bolus 10 min before the left circumflex coronary artery occlusion (OCC). Glibenclamide (0.3 mg/kg, i.v.) was administered as a bolus 20 min before the left circumflex coronary artery occlusion (glibenclamide pretreatment), or 2 min before reperfusion (glibenclamide posttreatment). Each point represents mean value. Bars indicate S.E.M. * $P < 0.05$, vs. Y-26763-treated group (two-way ANOVA, Tukey's test). Y, Y-26763; GL + Y, glibenclamide-pretreatment group before Y-26763 treatment; Y + GL, glibenclamide-posttreatment group after Y-26763 treatment.

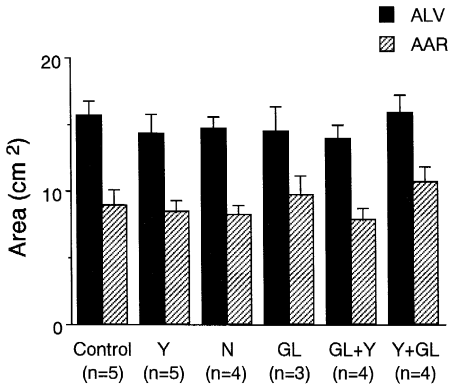

Fig. 3. The entire area of the left ventricle (ALV) and the area-at-risk (AAR) after digital image analysis with NIH Image software. Each point represents mean value. Bars indicate S.E.M. Y, Y-26763; N, nifedipine; GL, glibenclamide-alone; GL + Y, glibenclamide-pretreatment group before Y-26763 treatment; Y + GL, glibenclamide-posttreatment group after Y-26763 treatment.

Fig. 2 shows %SS in the ischemic region in dogs receiving Y-26763 with either pre- or post-treatment of glibenclamide (0.3 mg/kg, i.v.). When only glibenclamide was administered before the ischemic insults, it did not influence post-ischemic function depression as compared to control preparations (data not shown), but it completely abolished the salutary effect of Y-26763. When the same dose of glibenclamide was given 2 min before reperfusion, the recovery of regional contractile function produced by Y-26763 was not affected.

The entire area of left ventricle and the area-at-risk data are shown in Fig. 3. The areas-at-risk were almost the same in the experimental groups, and the entire area of left ventricles which included the areas-at-risk was also similar among groups.

4. Discussion

The present study strengthens and extends previously reported findings in an irreversible model of ischemia/reperfusion injury that the K^+ channel openers exert marked cardioprotective effects (Auchampach et al., 1991; Nakajima, 1992). Indeed, in a model of reversible ischemia reperfusion injury, the systemic administration of a nonhypotensive dose of Y-26763 resulted in a substantial, rapid recovery of postischemic myocardial wall function. The protective dose of Y-26763 did not substantially affect systemic and coronary hemodynamics. Thus, the recovery of postischemic regional contractile dysfunction by the

stunned myocardium cannot be attributed to a decrease in afterload and myocardial oxygen consumption during the occlusion period. In this experiment, the bolus injection of Y-26763 exerted a marked effect, although, in previous studies, drugs have been infused into the vein or coronary artery during the ischemic period (Gross et al., 1987a,b; Auchampach et al., 1992; D'Alomzo et al., 1992), indicating long duration of action of Y-26763.

This favorable effect of Y-26763 appears to be mediated by K_{ATP} channel activation which occurred during the ischemic insult in cardiac myocytes, because glibenclamide completely blocked the action of Y-26763 only when it was administered before the occlusion period. Glibenclamide is known to be a K_{ATP} channel blocker which has been shown to negate the salutary effects of various K_{ATP} channel openers in in vitro and in vivo models of ischemia/reperfusion damage (Auchampach et al., 1991; Ohta et al., 1991; Sargent et al., 1991). Thus, to protect the myocardium from a stunning episode, K_{ATP} channels need to be activated during or immediately before the ischemic period. Glibenclamide (0.3 mg/kg) itself did not modify the degree of stunning, no matter how it was injected before the ischemic insult. Moreover, glibenclamide had no effect on hemodynamics at such a low dose when administered alone or in combination with Y-26763. In the preliminary study, we confirmed that the same dose of glibenclamide slightly lowered blood glucose concentration at 10 min after the beginning of occlusion (79.7 ± 8.2 mg/dl, $n = 3$ from a baseline values of 112.7 ± 6.8 mg/dl), and at the end of reperfusion (70.3 ± 0.3 mg/dl) (unpublished). However, the antagonistic activity of this dose of glibenclamide occurred without alteration in the blood glucose levels, as reported (Auchampach et al., 1992). In fact, a higher dose of glibenclamide or the chemically related K_{ATP} channel antagonist tolbutamide has been reported to increase infarct size (Auchampach et al., 1991) and exacerbate stunning injury during reperfusion (Auchampach et al., 1992). However, their effects occurred independently of a change in collateral blood flow, and the ischemic bed size (Auchampach et al., 1992). And in this study, there was no remarkable difference in the area at risk in the left ventricle (%SS) in any experimental group. Taken together, all these results suggest that the cardioprotection appears to be due to the activation of myocardial K_{ATP} channels which occurs during ischemia and is independent of the peripheral or coronary blood flow.

Y-26763 produced minor effects on the hemodynamic parameters and the effects were similar to those of nifedipine. Nevertheless, only Y-26763 did account for the rapid recovery of postischemic myocardial segment function. The intrinsic mechanism by which myocardial K_{ATP} channel protects the heart against ischemia is not unveiled by the present study. The most often advanced explanation is that K_{ATP} channel opening, either in response to ischemia or by potassium channel openers, leads to a shortening of the plateau of the action potential (Fosset et al., 1988; Wilde et al., 1990) and thus reduces the open time of voltage-regulated calcium channels and reduces total cytosolic free calcium during the cardiac cycle (D'Alomzo et al., 1992). These events lead to a rapid decrease in contractile activity in the ischemic zone, and theoretically would preserve the activity of Na^+/Ca^{2+} exchanger in the calcium extrusion mode, thus delaying or preventing its reversal to calcium intrusion. Moreover, this mechanism may significantly contribute to calcium accumulation during ischemia (Cole, 1993). Recently, Smart et al. (1995) suggested that the inhibition of Na^+/Ca^{2+} exchange may be the mechanism of improved postischemic myocardial function, since amiloride induced complete recovery of systolic shortening, while this did not recover with hexamethylene amiloride (a specific inhibitor of Na^+/H^+ exchange) or nifedipine (a specific inhibitor of L-type Ca^{2+} channels) in the dog stunned myocardium model, indicating that the Ca^{2+} channel antagonist alone did not account for the improvement. This hypothesis is supported by the finding that Y-26763 accelerates the action potential shortening when injected into the coronary artery of hypo-oxygenated perfused hearts in open chest dogs, but nifedipine does not (Kawahara and Nakajima, 1994). A similar observation has been made by McPherson et al. (1993) in the isolated guinea-pig. However, in the same preparation, it has been reported that aprikalim can produce cardioprotection at a concentration which does not accelerate the loss of contractility at the onset of ischemia (Cavero, 1995; Cavero et al., 1996).

A recent report (Yao and Gross, 1994b) has clearly demonstrated that, in the barbital-anesthetized dog, injection of a hemodynamically ineffective dose of bimakalim can reduce myocardial infarct size without producing profibrillatory activity. Thus, the cardioprotective effects of K_{ATP} channel openers can be caused with doses that have no evident hemodynamic or profibrillatory activity. Nevertheless, K_{ATP} channel openers have the potential of facilitating ischemia-induced arrhythmia if they are used at sufficiently high doses (Grover et al., 1990). This might be a possible undesirable effect, if one considers that the opening of K_{ATP} channels within the ischemic region induces the shortening of action potential duration which may be a trigger for reentry arrhythmias, risking to culminate in ventricular fibrillation (Kantor et al., 1990). In contrast, since opening of K_{ATP} channels can prevent other types of arrhythmias, particularly those originating from abnormal automaticity or triggered activity (Opie, 1993), it remains to be seen whether the K_{ATP} channel opener will be proarrhythmic or antiarrhythmic.

Ischemic preconditioning (a brief period of ischemia followed by reperfusion) enhances the tolerance of cardiac myocytes to an ordinarily lethal ischemic insult, achieved by an initial brief exposure to ischemia (Kloner and Yellon, 1994). There are striking analogies between the cardioprotection afforded by K_{ATP} channel openers and pre-

conditioning since both are susceptible to glibenclamide blockade even in humans (Tomai et al., 1994). Myocardial stunning may occur in patients after a brief episode of ischemia such as angina pectoris, mild myocardial infarction, and minor (percutaneous transluminal coronary angioplasty) or major (cardiac valve replacement, heart transplantation) surgery. Therefore, protecting the myocardium from such a left ventricular dysfunction may be clinically useful.

Enhancing or accelerating myocardial K_{ATP} channel opening during ischemia with a potassium channel opener could be a novel effective therapeutic approach to treat ischemic heart disease. Escande and Cavero (1992) proposed that therapy with K_{ATP} channel openers may afford a permanent 'chemical preconditioning' which confers on the heart the extraordinary ability to better withstand transient oxygen deprivation, and consequently to suffer less damage during acute myocardial infarction. Y-26763 is the active metabolite of Y-27152 which is a potent antihypertensive agent with reduced tachycardiac effects (Nakajima, 1992; Nakajima et al., 1992). The cardioprotective property of Y-26763 confers a potential advantage on Y-27152 over other antihypertensive drugs if the findings reported in the animals are substantiated in humans. Additionally, the cardioprotective effects of Y-26763 against a stunning insult or against an ischemia with the potential to produce necrosis are also obtained with doses lacking hemodynamic effects. Future clinical studies with Y-26763 will prove whether activation of the myocardial K_{ATP} channel is a favorable mechanism affording protection to the human heart under an ischemic stress.

In conclusion, the present study demonstrates that Y-26763 improves recovery of myocardial segment function after a stunning insult without changing systemic and coronary hemodynamics. Glibenclamide negated the beneficial effect of Y-26763 only when it was administered before ischemia (not before starting reperfusion). Therefore, the cardioprotective effect of Y-26763 is mediated by activation of the myocardial K_{ATP} channel which appears to be involved in affording protection during ischemia and not at the reperfusion. In the future, potassium channel openers, 'preconditioning-mimetic' agents, may be applied therapeutically in, for instance, cardiopulmonary bypass, heart transplantation, angina and myocardial infarction.

Acknowledgements

The authors warmly thank Ms. Mizumi Goto and Mr. Toshio Hashimoto for statistical analyses, and Dr. Icilio Cavero for his careful critical reading of the manuscript.

References

Auchampach, J.A., M. Maruyama, I. Cavero and G.J. Gross, 1991, The new K channel opener aprikalim (RP 52891) reduces experimental infarct size in dogs in the absence of hemodynamic changes, J. Pharmacol. Exp. Ther. 259, 961.

Auchampach, J.A., M. Maruyama, I. Cavero and G.J. Gross, 1992, Pharmacological evidence for a role of ATP-dependent potassium channels in myocardial stunning, Circulation 86, 311.

Baxter, G.F. and D.M. Yellon, 1994, Ischaemic preconditioning of myocardium: a new paradigm for clinical cardioprotection?, Br. J. Clin. Pharmacol. 38, 381.

Braunwald, E. and R.A. Kloner, 1982, The stunned myocardium: prolonged, postischemic ventricular dysfunction, Circulation 66, 1146.

Cavero, I., 1995, Activation of cardiac ATP-sensitive K channels: a mechanism to reduce and delay ischemia-reperfusion damage, Heart Vessels Suppl. 10, 4.

Cavero, I., C. Hecquet, Y. Djellas, V. Gollot-Robert and M. Mestre, 1996, Activation of cardiac adenosine triphosphate-sensitive K^+ channels: an obligatory pathway for the cardioprotection afforded by ischemic and pharmacological preconditioning, in: Molecular and Cellular Mechanisms of Cardiovascular Regulation, eds. M. Endoh, M. Morad, H. Scholz and T. Iijima (Springer, Tokyo) p. 69.

Cole, W.C., 1993, ATP-sensitive K channels in cardiac ischemia: an endogenous mechanism for protection of the heart, Cardiovasc. Drugs Ther. 7, 527.

Cook, N.S., 1988, The pharmacology of potassium channels and their therapeutic potential, Trends Pharmacol. Sci. 9, 21.

D'Alomzo, A.J., R.B. Darbenzio, C.S. Parham and G.J. Grover, 1992, Effects of intracoronary cromakalim on postischemic contractile function and action potential duration, Cardiovasc. Res. 26, 1046.

Edwards, N.C., A.J. Sinusas, J.D. Bergin, D.D. Warson, M. Ruiz and G.A. Beller, 1992, Influence of subendocardial ischemia on transmural myocardial function, Am. J. Physiol. 262, H568

Escande, D. and I. Cavero, 1992, K channel opener and 'natural' cardioprotection, Trends Pharmacol. Sci. 13, 269.

Fosset, M., J.R. De Weille, R.D. Green, H. Schmid-Antomarchi and M. Lazdunski, 1988, Antidiabetic sulfonylureas control action potential properties in heart cells via high affinity receptors that are linked to ATP-dependent K^+ channels, J. Biol. Chem. 263, 7933.

Gross, G.J. and L.A. Auchampach, 1992, Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs, Circ. Res. 70, 223.

Gross, G.J., G.M. Pieper and D.C. Warltier, 1987a, Comparative effects of nicorandil, nitroglycerin, nicotinic acid, and SG-86 on the metabolic status and functional recovery of the ischaemic-reperfused myocardium, J. Cardiovasc. Pharmacol. 10 (Suppl. 8), S76.

Gross, G.J., D.C. Warltier and H.F. Hardman, 1987b, Comparative effects of nicorandil, a nicotinamide nitrate derivative, and nifedipine on myocardial reperfusion injury in dogs, J. Cardiovasc. Pharmacol. 10, 535.

Grover, G.J., P.G. Sleph and S. Dzwonczyk, 1990, Pharmacologic profile of cromakalim in the treatment of myocardial ischemia in isolated rat hearts and anesthetized dogs, J. Cardiovasc. Pharmacol. 16, 853.

Hartley, C.J., L.A. Latson, L.H. Michael, C.L. Seidel, R.M. Lewis and M.L. Entman, 1983, Doppler measurement of myocardial thickening with a single epicardial transducer, Am. J. Physiol. 245, H1066.

Kantor, P.F., W.A. Coetzee, E.E. Carmeliet, S.C. Dennis and L.H. Opie, 1990, Reduction of ischemic K loss and arrhythmias in rat hearts: effect of glibenclamide, a sulfonylurea, Circ. Res. 66, 478.

Kawahara, K. and T. Nakajima, 1994, Effect of a novel K channel opener Y-26763, an active metabolite of Y-27152, on contraction and monophasic action potential during normoxia in blood-perfused canine papillary muscle, Can. J. Physiol. Pharmacol. 72 (Suppl. 1), 92.

Kloner, B.A. and D.M. Yellon, 1994, Does ischemic preconditioning occur in patients?, J. Am. Coll. Cardiol. 24, 1133.

Lawson, C.S., 1994, Does ischaemic preconditioning occur in the human heart?, Cardiovasc. Res. 28, 1461.

McPherson, C.D., G.N. Pierce and W.C. Cole, 1993, Ischemic cardioprotection by ATP-sensitive K^+ channels involves high-energy phosphate preservation, Am. J. Physiol. 265, H1809.

Nakajima, T., 1992, Y-27152: a long-acting K^+ channel opener with less incidence of tachycardia, Cardiovasc. Drug Rev. 9, 372.

Nakajima, T., T. Shinohara, O. Yaoka, A. Fukunari, K. Shinagawa, K. Aoki, A. Katoh, T. Yamanaka, M. Setoguchi and T. Tahara, 1992, Y-27152, a long acting K^+ channel opener with less tachycardia: antihypertensive effects in hypertensive rats and dogs in conscious state, J. Pharmacol. Exp. Ther. 261, 730.

Noma, A., 1983, ATP-regulated K^+ channels in cardiac muscle, Nature 305, 147.

Ohta, H., Y. Jinno, K. Harada, N. Ogawa and H. Fukushima, 1991, Cardioprotective effects of KRN2391 and nicorandil on ischemic dysfunction in perfused rat heart, Eur. J. Pharmacol. 204, 171.

Opie, L.H., 1993, Modulation of ischemia by regulation of the ATP-sensitive potassium channel, Cardiovasc. Drugs Ther. 7, 507.

Pitsillides, K.F. and J.C. Longhurst, 1993, An ultrasonic system for measurement of absolute myocardial thickness using a single transducer, Am. J. Physiol. 268, H1358.

Rahman, F., A. Kato, K. Kawahara and T. Nakajima, 1996, Y-26763 protects the working rat myocardium from ischemia/reperfusion injury through opening of K_{ATP} channels, Eur. J. Pharmacol. 317, 293.

Sargent, C.A., M.A. Smith, S. Dzwonczyk, P.G. Sleph and G.J. Grover, 1991, Effect of potassium channel blockade on the anti-ischemic actions of mechanistically diverse agents, J. Pharmacol. Exp. Ther. 259, 97.

Smart, S.C., A. LoCurto, J.E. Schults, K.B. Sagar and D.C. Warltier, 1995, Intracoronary amiloride prevents contractile dysfunction of postischemic 'stunned' myocardium: role of hemodynamic alterations and inhibition of Na^+/H^+ exchange and L-type Ca^{2+} channels, J. Am. Coll. Cardiol. 26, 1365.

Tomai, F., F. Crea, A. Gaspardone, F. Versaci, R. DePaulis, A. Penta de Peppo, L. Chiariello and P.A. Gioffre, 1994, Ischemic preconditioning during coronary angioplasty is prevented by glibenclamide, a selective ATP-sensitive K^+ channel blocker, Circulation 90, 700.

Wilde, A.A.M., D. Escande, C.A. Shumacher, D. Thuringer and M. Mestre, 1990, Potassium accumulation in the globally ischemic mammalian heart: a role for the ATP-sensitive potassium channel, Circ. Res. 67, 835.

Yao, Z. and G.J. Gross, 1994a, Activation of ATP-sensitive potassium channels lowers threshold for ischemic preconditioning in dogs, Am. J. Physiol. 267, H1888.

Yao, Z. and G.J. Gross, 1994b, Effects of the K_{ATP} channel opener bimakalim on coronary blood flow, monophasic action potential duration, and infarct size in dogs, Circulation 89, 1769.

Yellon, D.M., A.M. Alkhulaifi and W.B. Pugsley, 1993, Preconditioning the human myocardium, Lancet 342, 276.