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Absence of exacerbation of myocardial stunning in anesthetized dogs treated with KAD-1229, a novel hypoglycemic agent

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Abstract

The effect of (+)-momocalcium bis[(2*S*,3*a*,7*a-cis*)-α-benzylhexahydro-γ-oxo-2-isoindolinebutyrate]dihydrate (KAD-1229), a novel hypoglycemic agent with a chemical structure different from that of the sulfonylureas, on myocardial stunning was assessed in anesthetized dogs by comparison with that of glibenclamide, a sulfonylurea. Even though their hypoglycemic effects were of similar magnitude, glibenclamide (1 mg/kg, i.v.), but not KAD-1229, exacerbated the myocardial stunning induced by occlusion/reperfusion of the descending coronary artery. In a receptor-binding experiment, unlabeled glibenclamide completely inhibited [^{3}H]glibenclamide binding to the myocardium, but KAD-1229 did not. These results suggest that the difference in binding properties of KAD-1229 and glibenclamide toward cardiac sulfonylurea receptors is one of the causes of their different effects on myocardial stunning. It is likely that KAD-1229 is highly specific for pancreatic sulfonylurea receptors and is speculated to be a safer hypoglycemic agent than, at least, glibenclamide.

Keywords: KAD-1229; Glibenclamide; Myocardial stunning; Sulfonylurea receptor; Diabetes mellitus

1. Introduction

Sulfonylureas, which are widely used for the treatment of diabetes mellitus, release insulin from pancreatic β-cells by closing ATP-dependent K^+ channels. At the molecular level, the ATP-dependent K^+ channel is thought to be a complex of a binding site for sulfonylureas (sulfonylurea receptor: SUR) and an inward-rectifying K^+ channel (K_{IR}). The subtypes of sulfonylurea receptor and/or inward-rectifying potassium channels are known to differ tissue by tissue: SUR1 with K_{IR} 6.2 in the pancreas, SUR2A with K_{IR} 6.2 in the heart, and SUR2B with K_{IR} 6.1 in vascular smooth muscle (Yokoshiki et al., 1998). Although the roles played by these ATP-dependent K^+ channels (except the pancreatic type) are uncertain, opening of the cardiac type may participate in cytoprotection during ischemia/reperfusion (Terizic et al., 1995). In fact, several K^+ channel openers have been reported to improve functional and metabolic recovery in ischemic rat hearts, while glibenclamide, a sulfonylurea, has been found to block the beneficial effects of these agents (Grover et al., 1990). Glibenclamide has also been reported to impair electrical and mechanical recovery in guinea pig ventricular walls (Cole et al., 1991) and myocardial segment shortening in dogs (Auchampach et al., 1992) after ischemia/reperfusion.

(+)-Momocalcium bis[(2*S*,3*a*,7*a-cis*)-α-benzylhexahydro-γ-oxo-2-isoindolinebutyrate] dihydrate (KAD-1229), which has a chemical structure different from that of the sulfonylureas, is a novel rapid-acting and short-lasting hypoglycemic agent with an insulinotropic action (Misawa et al., 2001; Ohnota et al., 1995; Ohnota et al., 1994). The principal mechanism underlying the insulinotropic action of KAD-1229 is similar to that described for the sulfonylureas; binding to pancreatic sulfonylurea receptors and closing ATP-dependent K^+ channels (Mogami et al., 1994; Ohnota et al., 1994). In view of this similarity in their principal mechanism of action, the harmful effects of KAD-1229 on patients with ischemic heart disease might be expected to be considerable, as they are in the case of the sulfonylureas. However, the effects of KAD-1229 on cardiac function after ischemia/reperfusion have not yet

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been reported upon. In this study, we compared the effect of KAD-1229 on myocardial stunning in anesthetized dogs with that of glibenclamide. Having found differences in their effects, we then examined the affinities of these drugs for the sulfonylurea receptors in the canine myocardium to try to establish the mechanistic reason for these differences.

2. Materials and methods

2.1. Materials

KAD-1229 was synthesized by Shiono Finesse (Fukui, Japan) and glibenclamide was purchased from Sigma (St.

Table 1
Hemodynamic data

	LVSP (mm Hg)	LVDP (mm Hg)	SBP (mm Hg)	DBP (mm Hg)	HR (beats/min)
Pre-treatment					
Vehicle	117.8 ± 7.4	6.1 ± 1.0	115.2 ± 6.0	85.8 ± 4.7	132.2 ± 7.9
KAD 0.003 mg/kg	123.3 ± 3.4	3.3 ± 1.8	128.3 ± 4.4	97.3 ± 4.4	155.0 ± 7.8
KAD 0.03 mg/kg	129.8 ± 9.4	4.0 ± 2.1	133.8 ± 9.8	95.5 ± 6.6	135.3 ± 5.2
KAD 0.3 mg/kg	118.4 ± 4.8	2.3 ± 1.0	123.5 ± 4.6	93.0 ± 4.0	136.2 ± 2.7
KAD 3 mg/kg	116.7 ± 4.9	2.9 ± 0.8	125.7 ± 4.1	93.7 ± 3.7	131.2 ± 10.8
GB 0.01 mg/kg	129.1 ± 7.8	4.3 ± 1.2	132.5 ± 7.8	100.3 ± 6.2	143.5 ± 8.4
GB 0.1 mg/kg	121.4 ± 4.8	7.0 ± 3.6	124.3 ± 6.9	92.5 ± 5.4	126.2 ± 6.7
GB 1 mg/kg	126.3 ± 4.8	3.7 ± 1.1	130.2 ± 4.5	97.8 ± 2.9	152.3 ± 7.0
Reperfusion (0 min)					
Vehicle	107.4 ± 5.4	9.6 ± 1.3	111.5 ± 5.8	82.7 ± 4.5	132.2 ± 8.1
KAD 0.003 mg/kg	117.3 ± 3.9	4.6 ± 1.7	122.8 ± 4.6	94.2 ± 4.8	153.5 ± 8.5
KAD 0.03 mg/kg	129.7 ± 9.0	7.9 ± 2.8	135.3 ± 8.9	96.5 ± 6.2	134.0 ± 5.3
KAD 0.3 mg/kg	116.8 ± 6.2	4.8 ± 1.3	121.2 ± 6.0	91.3 ± 5.1	137.3 ± 4.5
KAD 3 mg/kg	117.2 ± 5.4	4.4 ± 0.9	125.8 ± 4.3	95.2 ± 4.4	129.5 ± 10.3
GB 0.01 mg/kg	125.2 ± 9.1	9.3 ± 1.5	127.7 ± 9.2	96.3 ± 7.3	141.5 ± 8.5
GB 0.1 mg/kg	121.5 ± 5.5	10.3 ± 3.8	123.2 ± 7.3	92.3 ± 6.1	125.8 ± 7.4
GB 1 mg/kg	125.4 ± 5.5	7.4 ± 1.9	131.7 ± 5.6	101.7 ± 3.6	149.7 ± 7.4
Reperfusion (15 min)					
Vehicle	108.4 ± 5.8	7.3 ± 1.0	111.8 ± 5.0	81.3 ± 4.6	131.8 ± 8.8
KAD 0.003 mg/kg	116.8 ± 2.5	3.1 ± 1.7	122.3 ± 4.0	92.7 ± 3.6	151.2 ± 7.7
KAD 0.03 mg/kg	125.1 ± 8.2	2.1 ± 2.4	133.7 ± 8.7	94.7 ± 6.0	133.7 ± 5.7
KAD 0.3 mg/kg	115.2 ± 4.0	1.9 ± 0.8	120.8 ± 4.4	89.3 ± 3.2	135.5 ± 3.0
KAD 3 mg/kg	115.9 ± 4.5	3.5 ± 0.8	122.7 ± 4.6	90.8 ± 4.6	129.5 ± 10.1
GB 0.01 mg/kg	121.0 ± 6.8	5.8 ± 1.5	125.5 ± 6.0	92.7 ± 4.5	138.7 ± 7.9
GB 0.1 mg/kg	118.9 ± 4.7	8.3 ± 3.5	122.2 ± 7.4	89.7 ± 6.0	123.3 ± 7.4
GB 1 mg/kg	121.3 ± 6.1	5.1 ± 1.5	128.5 ± 5.6	96.7 ± 3.2	147.2 ± 7.6
Reperfusion (60 min)					
Vehicle	114.7 ± 6.6	7.1 ± 0.8	116.2 ± 5.1	85.2 ± 4.0	131.8 ± 8.9
KAD 0.003 mg/kg	116.7 ± 3.2	3.1 ± 1.7	123.2 ± 3.7	92.5 ± 2.2	149.2 ± 6.5
KAD 0.03 mg/kg	125.3 ± 7.2	4.6 ± 2.5	131.5 ± 7.0	93.3 ± 4.6	131.7 ± 5.2
KAD 0.3 mg/kg	115.3 ± 2.6	1.3 ± 0.8	123.8 ± 2.8	91.2 ± 2.2	135.5 ± 1.5
KAD 3 mg/kg	117.7 ± 3.8	4.0 ± 1.0	125.2 ± 3.2	90.7 ± 3.0	132.5 ± 10.0
GB 0.01 mg/kg	122.7 ± 7.8	5.6 ± 1.2	126.2 ± 6.9	93.7 ± 6.0	136.8 ± 8.0
GB 0.1 mg/kg	120.2 ± 5.9	9.3 ± 4.0	124.3 ± 7.8	90.7 ± 6.4	122.7 ± 7.0
GB 1 mg/kg	120.3 ± 6.3	5.3 ± 1.6	125.2 ± 5.2	91.3 ± 3.8	147.2 ± 5.6
Reperfusion (240 min)					
Vehicle	113.7 ± 7.0	5.8 ± 1.2	116.7 ± 5.9	84.3 ± 4.7	140.3 ± 7.3
KAD 0.003 mg/kg	113.3 ± 5.7	2.4 ± 1.6	120.0 ± 5.1	85.0 ± 4.6	158.8 ± 7.3
KAD 0.03 mg/kg	124.5 ± 4.3	3.6 ± 2.3	130.0 ± 4.1	89.3 ± 2.9	140.3 ± 5.2
KAD 0.3 mg/kg	112.6 ± 3.1	1.1 ± 0.6	123.2 ± 3.5	89.5 ± 3.2	137.2 ± 2.1
KAD 3 mg/kg	118.3 ± 4.8	3.8 ± 1.7	126.0 ± 4.4	90.8 ± 4.1	137.5 ± 9.3
GB 0.01 mg/kg	123.3 ± 7.1	4.3 ± 0.9	128.2 ± 7.2	91.5 ± 5.7	140.2 ± 8.9
GB 0.1 mg/kg	121.6 ± 4.0	8.8 ± 3.4	124.8 ± 6.5	89.5 ± 4.6	130.0 ± 6.0
GB 1 mg/kg	121.3 ± 3.4	5.8 ± 1.2	126.7 ± 3.3	91.3 ± 2.9	150.2 ± 4.2

Data are presented as the means ± S.E.M. from six animals.
KAD, KAD-1229; GB, glibenclamide; LVSP, left ventricular systolic pressure; LVDP, left ventricular diastolic pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate.

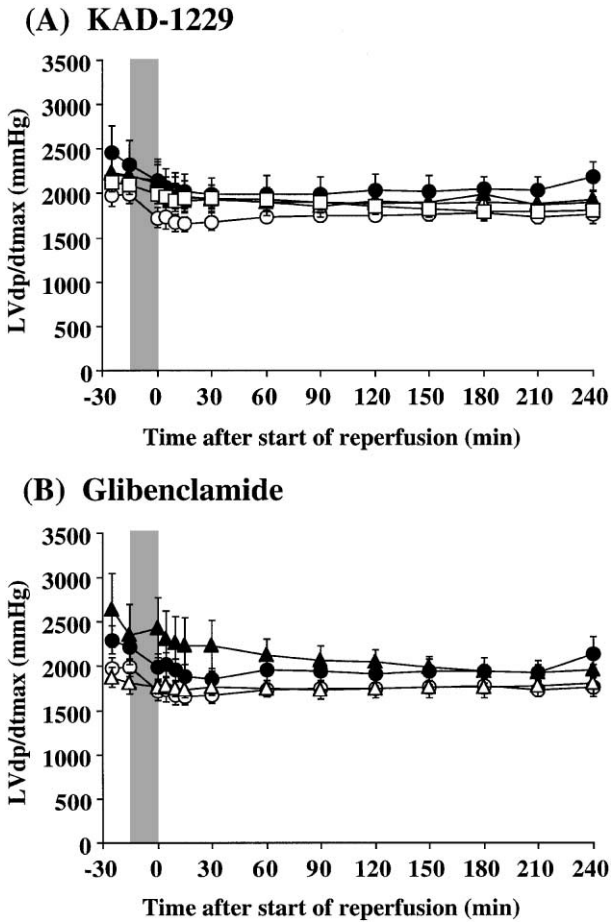

Fig. 1. Effects of KAD-1229 (A) and glibenclamide (B) on change in maximum first derivative of left ventricular pressure ($LVdp/dt_{max}$) during ischemia/reperfusion. In A, vehicle: open circle; KAD-1229: closed circle, 0.003 mg/kg; open triangle, 0.03 mg/kg; closed triangle, 0.3 mg/kg; open square, 3 mg/kg. In B, vehicle: open circle; glibenclamide: closed circle, 0.01 mg/kg; open triangle, 0.1 mg/kg; closed triangle, 1 mg/kg. Each value represents the mean ± S.E.M from six animals. Vehicle, KAD-1229, or glibenclamide was injected 25 min before the start of reperfusion. Hatched vertical column indicates the ischemic period (15 min).

Louis, MO, USA). Both were dissolved in 100% polyethylene glycol 400 (Wako, Osaka, Japan) for the in vivo study and in dimethyl sulfoxide (Nakarai Tesque, Kyoto, Japan) for the in vitro study. The doses and concentrations used in this study were based on those producing adequate hypoglycemic effects in animals and adequate insulin release from pancreatic β-cells. Other chemicals were obtained from commercial sources.

2.2. Methods

2.2.1. Animals

Mongrel dogs of either sex weighing about 15 kg were obtained from Kitayama Labes (Nagano, Japan). The animals were housed individually with free access to tap water and commercial food pellets (CD-5; Nihon Crea, Osaka, Japan). The room temperature and humidity were about 23 °C and 55%, respectively, and a 12-h light/dark cycle was maintained. The experiments in this study were approved by the Kissei Pharmaceutical Animal Care and Use Committee.

2.2.2. Assessment of hemodynamics, coronary blood flow, and myocardial segment shortening

Anesthesia was induced and maintained using sodium pentobarbital (30 mg/kg, i.v. and 3–5 mg/kg/h, i.v., respectively). The animal was artificially ventilated using a volume-limited ventilator (SN-480-3; Shinano-Seisakusho, Tokyo, Japan) via a tracheal cannula. Body temperature

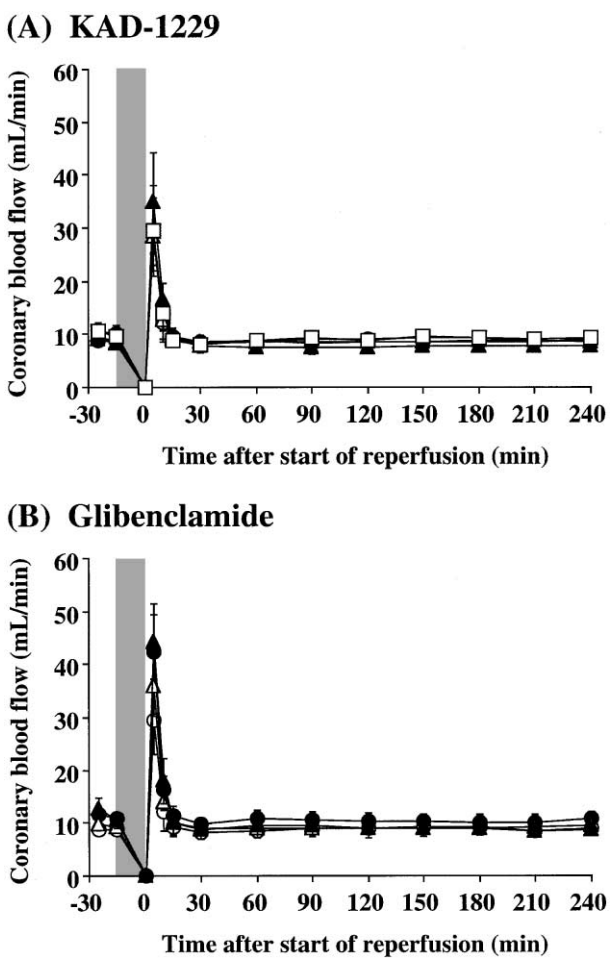

Fig. 2. Effects of KAD-1229 (A) and glibenclamide (B) on change in coronary blood flow during ischemia/reperfusion. In A, vehicle: open circle; KAD-1229: closed circle, 0.003 mg/kg; open triangle, 0.03 mg/kg; closed triangle, 0.3 mg/kg; open square, 3 mg/kg. In B, vehicle: open circle; glibenclamide: closed circle, 0.01 mg/kg; open triangle, 0.1 mg/kg; closed triangle, 1 mg/kg. Each value represents the mean ± S.E.M from six animals. Vehicle, KAD-1229, or glibenclamide was injected 25 min before the start of reperfusion. Hatched vertical column indicates the ischemic period (15 min).

was maintained at 37 ± 1 °C. Catheters were inserted into the femoral artery and femoral vein for blood pressure monitoring and for blood sampling and drug administration, respectively. The electrocardiogram (surface lead II) was monitored continuously and heart rate was obtained from it. A catheter-tip transducer (5-Fr; Millar Instrument, Houston, USA) was inserted into the left ventricle via the left carotid artery for the measurement of left ventricular pressure, from which the first derivative of left ventricular pressure ($LVdp/dt$) was derived. The left ventricular wall was exposed via a thoracotomy, and the main trunk of the left anterior descending coronary artery was dissected free from adjacent tissues. The artery was loosely encircled with a silk thread at a position distal to the first diagonal branch. For the measurement of myocardial-segment shortening, a pair of ultrasonic crystals (Segment Length; Sonotec, San Diego, CA, USA) was implanted approximately 1 cm apart within the perfusion area of the descending coronary artery. The length of each segment in the diastolic and systolic phases was determined by taking the segment length at the beginning of the positive phase of positive first derivative of left ventricular pressure and that at nadir, respectively. To obtain percentage segment shortening, the change in segment length (diastolic length minus systolic length) was expressed as a percentage of diastolic segment length. The blood flow in the descending

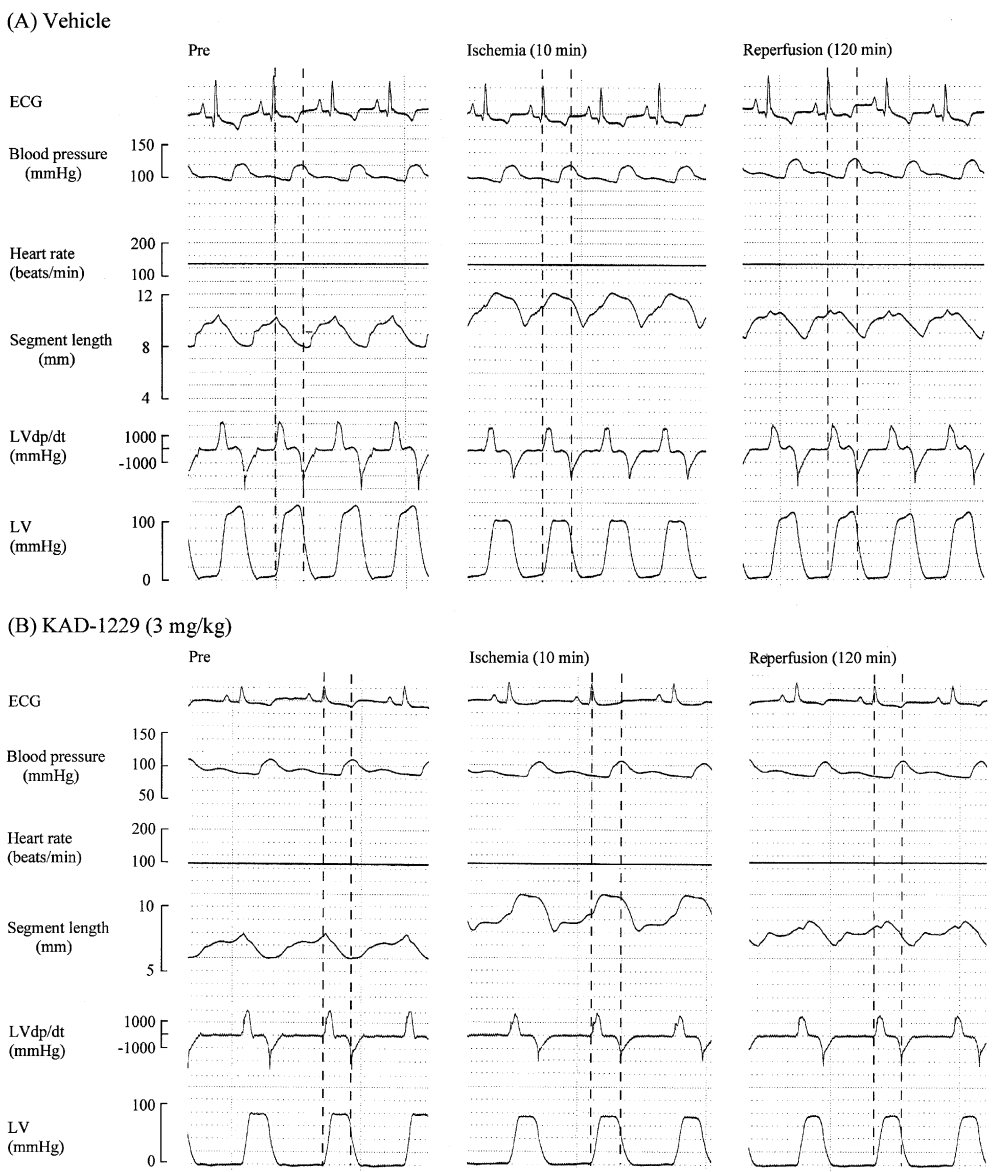

Fig. 3. Typical traces from vehicle- (A), KAD-1229- (B), and glibenclamide- (C) treated groups. Vertical dotted lines indicate the time of determination of diastolic and systolic segment length at the beginning of the positive phase of positive first derivative of left ventricular pressure and at its negative peak, respectively. ECG: electrocardiogram (surface lead II); $LVdp/dt$: the first derivative of left ventricular pressure; LV: left ventricular pressure; Pre: before drug treatment and ischemia.

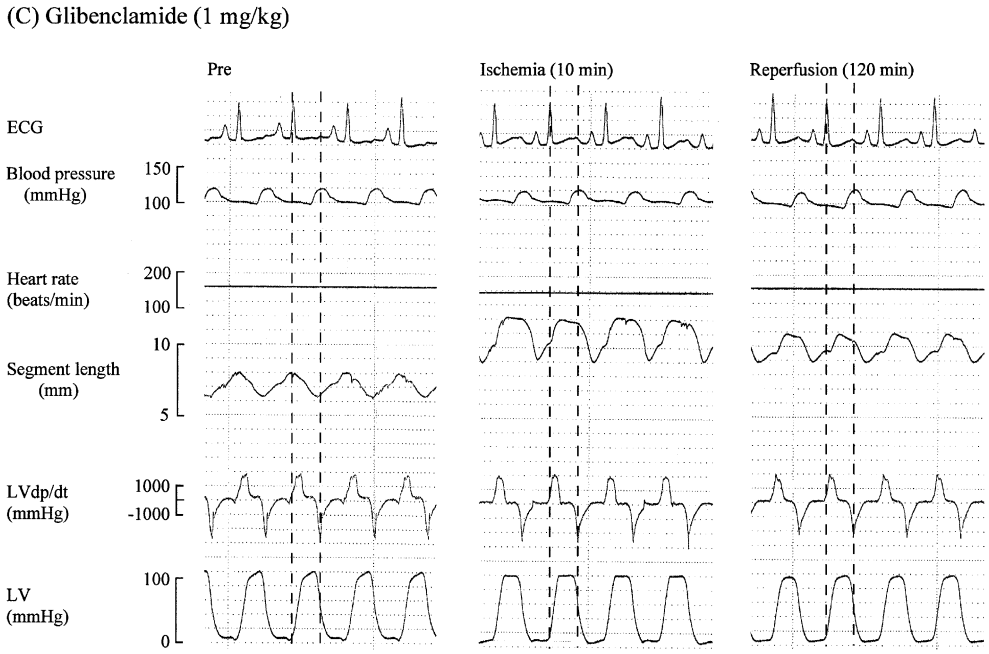

Fig. 3 (*continued*).

coronary artery was measured at a position proximal to the loose ligature using an ultrasonic flow meter (T106; Transonic, New York, USA). Myocardial ischemia, which was initiated by tightening the ligature around the descending coronary artery, was maintained for 15 min. Drugs were administered intravenously 10 min before the initiation of ischemia.

2.2.3. Assessment of plasma glucose level

Blood samples from the femoral vein were collected into heparinized tubes, the plasma was separated, and its glucose concentration was measured with a commercial assay kit (Glucose B-test; Wako, Osaka, Japan). The area under the curve for plasma glucose was calculated from these data.

2.2.4. Assessment of binding to myocardial sulfonylurea receptors

The binding assay was performed using a method described previously (Schmid-Antomarchi et al., 1987; French et al., 1990) with some modifications. For the preparation of myocardial membranes, dogs were killed by exsanguination under pentobarbital anesthesia (35 mg/kg, i.v.). The left ventricle was immediately removed and submerged in ice-cold Krebs–Henseleit solution (pH 7.4). After homogenization, the tissue was sonicated (5 s, 5 times with a 1-s cooling interval) in ice-cold 40 mM HEPES/NaOH buffer (pH 7.4) and then centrifuged at $1000 \times g$ for 10 min at 4 °C. The supernatant was centrifuged at $100\,000 \times g$ for 60 min at 4 °C, and the pellet was suspended in a 20 mM HEPES/NaOH buffer. The protein content was measured using a commercial assay kit (BCA™ protein assay kit; Rockford, IL, USA).

For the displacement assay, 200 μg of myocardial membranes in 500 μl was incubated with 0.8 nM of [^{3}H]glibenclamide plus various concentrations of KAD-1229 or glibenclamide for 2 h at 20 °C. The incubation was terminated by rapid filtration through a Whatman GF/C filter (Whatman, Maidstone, England) followed by rinsing with cold 50 mM Tris/HCl buffer (pH 7.4). The radioactivity was measured with a liquid scincillation counter (Tricarb1900; Packard, Research Parkway Meriden, CT, USA).

2.2.5. Statistical analysis

A two-way analysis of variance was used to make multiple comparisons among groups (hemodynamics, segment shortening, and plasma glucose level). A one-way analysis of variance was used for the data concerned, obtained in respect to the area under the plasma-glucose curve. When a significant difference was detected, the data were further analyzed using Dunnett's test. A P value less than 0.05 was considered to indicate statistical significance. StatView software (ver. 5.0; Abacus Concepts, Berkeley, CA, USA) was used for the statistical analysis.

3. Results

3.1. Hemodynamics and coronary blood flow

In the vehicle-treated group, ischemia/reperfusion led to only slight changes in the maximum first derivative of

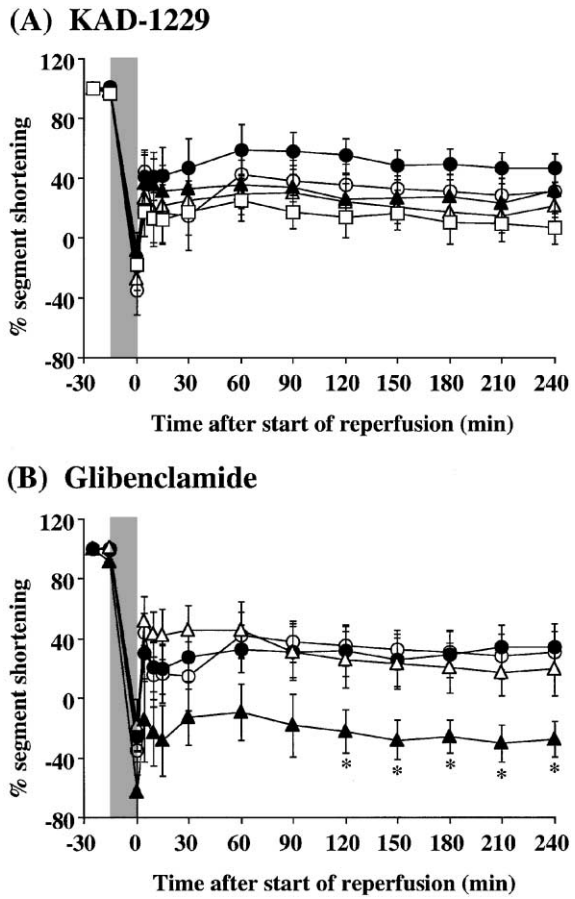

Fig. 4. Effects of KAD-1229 (A) and glibenclamide (B) on percentage segment shortening during ischemia/reperfusion. In A, vehicle: open circle; KAD-1229: closed circle, 0.003 mg/kg; open triangle, 0.03 mg/kg; closed triangle, 0.3 mg/kg; open square, 3 mg/kg. In B, vehicle: open circle; KAD-1229: closed circle, 0.01 mg/kg; open triangle, 0.1 mg/kg; closed triangle, 1 mg/kg. Each value represents the mean ± S.E.M from six animals. Vehicle, KAD-1229, or glibenclamide was injected 25 min before the start of reperfusion. Hatched vertical column indicates the ischemic period (15 min). * $P < 0.05$ vs. vehicle-treated group at the same time.

left ventricular pressure ($LVdp/dt_{max}$). Neither KAD-1229 nor glibenclamide had any significant effect on the changes in this parameter (Fig. 1). Likewise, left ventricular diastolic pressure, left ventricular systolic pressure, systemic blood pressure, and heart rate were less affected by ischemia/reperfusion, and neither drug had any significant effect on the changes (Table 1). In vehicle-treated animals, the coronary blood flow was elevated above the pre-ischemic value for 5 min or so after the start of reperfusion, indicating reactive hyperemia, and the pre-ischemic level was maintained thereafter. Neither drug had any significant effect on this pattern of changes (Fig. 2).

3.2. Myocardial-segment shortening

Typical traces from vehicle-, KAD-1229- and glibenclamide-treated groups are shown in Fig. 3. In every group, diastolic segment length was longer than systolic segment length before ischemia (Pre), and this trend was reversed during ischemia (systolic segment length became longer than diastolic one). After the start of reperfusion, diastolic and segment lengths began to recover to the pre-ischemic level trend in the vehicle- and KAD-1229-treated groups. However, no such recovery was observed in the glibenclamide-treated group.

The percentage segment shortening obtained in all animals is shown in Fig. 4. There was no significant difference in the values obtained for percentage segment shortening before the induction of myocardial ischemia among the vehicle-, KAD-1229- and glibenclamide-treated groups. In the vehicle-treated group, the percentage segment shortening decreased markedly during ischemia, and even reached negative values. Recovery toward the pre-ischemic value began immediately after coronary reperfusion, but

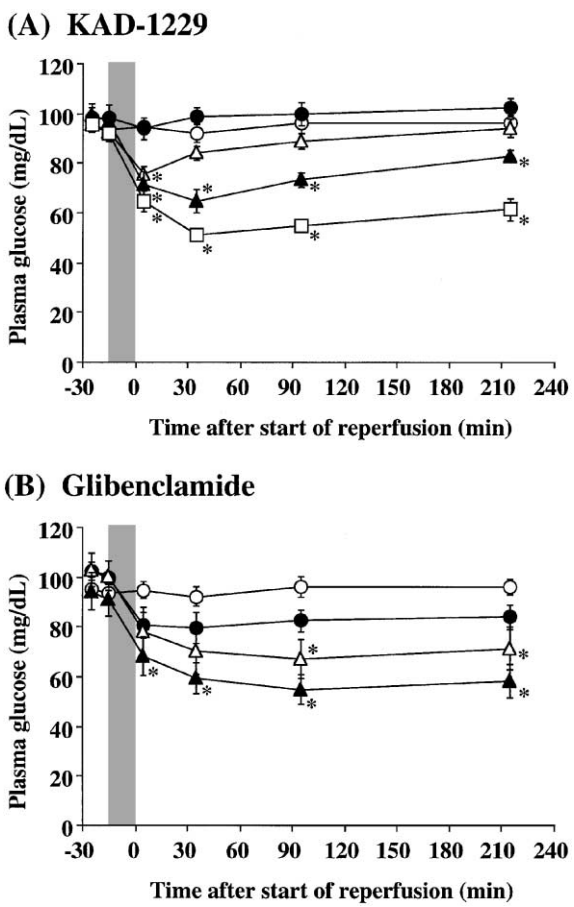

Fig. 5. Effects of KAD-1229 (A) and glibenclamide (B) on plasma blood glucose. In A, vehicle: open circle; KAD-1229: closed circle, 0.003 mg/kg; open triangle, 0.03 mg/kg; closed triangle, 0.3 mg/kg; open square, 3 mg/kg. In B, vehicle: open circle; glibenclamide: closed circle, 0.01 mg/kg; open triangle, 0.1 mg/kg; closed triangle, 1 mg/kg. Each value represents the mean ± S.E.M from six animals. Vehicle, KAD-1229, or glibenclamide was injected 25 min before the start of reperfusion. Hatched vertical column indicates the ischemic period (15 min). * $P <$ 0.05 vs. vehicle-treated group at the same time.

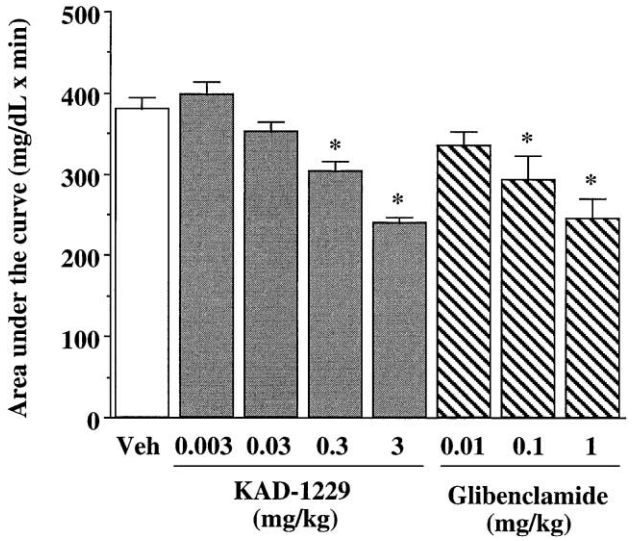

Fig. 6. Effects of KAD-1229 and glibenclamide on the area under the curve for plasma glucose after drug administration. Area under the curve was calculated from the data shown in Fig. 5. Each column represents the mean ± S.E.M from six animals. * $P < 0.05$ vs. vehicle-treated group. Veh: vehicle.

was incomplete. KAD-1229 (0.003, 0.03, 0.3, and 3 mg/kg) had no significant effect on this pattern of changes. Glibenclamide, at 0.01 and 0.1 mg/kg, also had no effect. However, at the highest dose used, 1 mg/kg, it markedly impaired recovery—the difference from the vehicle-treated group being significant at 120–240 min (the end of the study period) after the start of coronary reperfusion.

3.3. Plasma glucose level

KAD-1229 (0.03, 0.3, 3 mg/kg) and glibenclamide (0.1 and 1 mg/kg) dose dependently and significantly decreased the plasma glucose level (Fig. 5). The areas under the curve obtained for the KAD-1229-treated group at 0.03, 0.3, and 3 mg/kg were comparable with those obtained for the glibenclamide-treated group at 0.01, 0.1, and 1 mg/kg, respectively (Fig. 6).

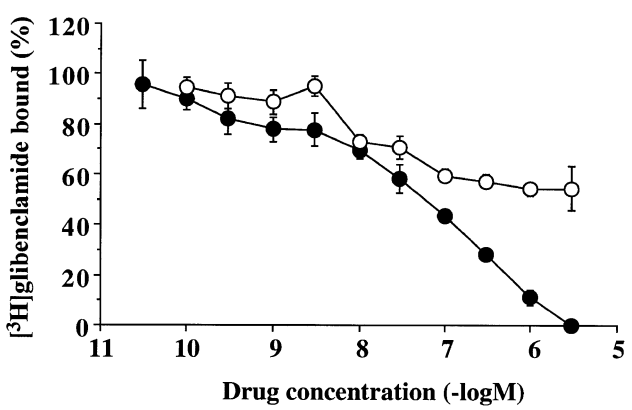

Fig. 7. Inhibition of [^{3}H]glibenclamide binding to myocardial membranes by KAD-1229 and glibenclamide. Open circle and closed circle indicate values obtained following KAD-1229 and glibenclamide-treatment, respectively. Each value represents the mean ± S.E.M from three to four experiments.

3.4. Displacement of [^{3}H]glibenclamide from myocardial sulfonylurea receptors

The displacement curves obtained for the effects of KAD-1229 and glibenclamide on the binding of [^{3}H]glibenclamide to myocardial sulfonylurea receptors (Fig. 7) show that glibenclamide almost completely displaced [^{3}H]glibenclamide, whereas with KAD-1229, displacement was less than 50% even at the highest concentration used.

4. Discussion

Coronary heart disease is one of the complications of diabetes mellitus, and the prevalence is higher in diabetics than in normal subjects (Haffner et al., 1998; Yudkins and Hendra, 1992; Kannel and McGee, 1979). Sulfonylureas are widely used for the treatment of diabetes mellitus; however, a much-discussed question is whether, in clinical use, sulfonylureas have a harmful effect on patients with ischemic heart disease (Jollis et al., 1999; Engler., 1998; Brady et al., 1998; Cleveland et al., 1997; Leibowitz and Cerasi, 1996). Thus, in the development of antidiabetic agents, assessment of the harmful effects on the cardiac system is important. Glibenclamide has been reported to worsen the recovery from myocardial stunning (Auchampach et al., 1992); thus, we assessed the effect of KAD-1229 on stunning, comparing it with that of glibenclamide in this study.

Although the hypoglycemic effects of KAD-1229 and glibenclamide at the doses we now used were of the same magnitude, recovery from the decrease in percentage segment shortening was suppressed by glibenclamide (at the highest dose used) but not by KAD-1229. These results indicated that glibenclamide can worsen myocardial stunning as previously reported (Auchampach et al., 1992), while KAD-1229 has no effect. The mechanisms underlying the effects of KAD-1229 and glibenclamide are the same: closure of ATP-dependent K^+ channels following binding to sulfonylurea receptors. It is known that the sulfonylurea receptor subtypes associated with ATP-dependent potassium channels differ between the pancreas and heart (Yokoshiki et al., 1998). For this reason, we thought that a difference in affinity for cardiac sulfonylurea receptors could well account for the differing effect of these two drugs on myocardial stunning. In fact, the results of the present displacement assay showed that KAD-1229 bound to the cardiac sulfonylurea receptors less than did glibenclamide. It has previously been reported that KAD-1229 completely inhibits the binding of [^{3}H]-glibenclamide to

the sulfonylurea receptors on HIT T15 cells, a hamster pancreatic β-cell line (Ohnota et al., 1994). Taken together, the above results suggest that KAD-1229 and glibenclamide differ in their binding profile toward pancreatic and cardiac sulfonylurea receptors and that this may account, at least in part, for the difference in their effects (both having a hypoglycemic effect, but only glibenclamide exacerbate myocardial stunning).

Myocardial stunning per se is considered to be an innocuous clinical entity (Heusch, 1998; Duncker et al., 1998), and the results obtained from the present study do not directly demonstrate the clinical safety of KAD-1229 in the cardiac system of diabetic patients. The mechanism of the worsening effect of glibenclamide on myocardial stunning appears to be through blockade of cardiac K_{ATP} channels (Del Valle et al., 2001). Moreover, opening of the cardiac K_{ATP} channels is thought to play a part in cytoprotection during ischemia/reperfusion (Terizic et al., 1995). Therefore, from the results obtained in the present study, it may be speculated that KAD-1229 is a safer hypoglycemic agent than, at least, glibenclamide.

Recently, a number of studies on myocardial ATP-dependent K^+ channels have been published. These studies have (i) elucidated the importance of such channels in the cardioprotective effect of ischemic preconditioning, (ii) distinguished mitochondrial from sarcolemmal channels, and (iii) established that glibenclamide blocks both sarcolemmal and mitochondrial ATP-dependent K^+ channels and prevents the cardioprotective effect of preconditioning (O'Rourke, 2000; Gross and Fryer, 1999; Szewczyk and Marbán, 1999). The results obtained from the present study raise the possibility that affinity for mitochondrial ATP-dependent K^+ channels and prevention of the cardioprotective effect of preconditioning may both be weaker during KAD-1229 treatment than during glibenclamide treatment. However, additional studies will be needed to clarify this point.

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