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Effect of glibenclamide on ischemic canine myocardium with glucose infusion

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Abstract

Glibenclamide, an ATP-sensitive K (K_{ATP}) channel blocker, worsens the ischemia-induced metabolic derangement in the heart through inhibition of K_{ATP} channels. We examined whether the hypoglycemic effect of glibenclamide was involved in the worsening of myocardial energy metabolism during ischemia. Pentobarbital-anesthetized dogs were subjected to 15-min ligation of the left anterior descending coronary artery. Either vehicle (dimethyl sulfoxide, DMSO) or glibenclamide (1 mg/kg) was injected i.v. 10 min before the ligation. In half of the animals given glibenclamide, glucose was continuously infused at 3 mg/kg per min immediately after glibenclamide injection. Glibenclamide increased the serum insulin level and decreased the blood glucose level. Glucose infusion completely abolished the hypoglycemia due to glibenclamide. Glibenclamide enhanced the decrease in ATP and total adenine nucleotides and increase in tissue lactate caused by ischemia. Glucose infusion did not cancel the augmentation of ischemia-induced alterations of myocardial energy metabolism caused by glibenclamide. These results suggest that K_{ATP} channels directly play an important role in endogenous mechanisms of myocardial protection against ischemic damage.

Keywords: K_{ATP} channel; Myocardial ischemia; Glucose; Glibenclamide

1. Introduction

Adenosine triphosphate (ATP)-sensitive K (K_{ATP}) channels are primarily modulated by the intracellular level of ATP. These channels have been identified in various organs and tissues, including cardiac muscle (Noma, 1983), pancreatic β cells (Cook and Hales, 1984), and vascular smooth muscle (Standen et al., 1989). Both pancreatic β cells and myocardial cells possess K_{ATP} channels which belong to Type 1 as classified by Ashcroft and Frances (1990). In the heart, ischemia leads to activation of the K_{ATP} channel due to reduction of the tissue ATP level in the myocardium and coronary smooth muscle (Cole et al., 1991). Because the activation of K_{ATP} channels reduces Ca^{2+} influx through the voltage-dependent Ca^{2+} channels, it decreases myocardial contractility and dilates the coronary artery (Barry, 1991). The former decreases myocardial energy demand and the latter increases energy supply. Therefore, K_{ATP} channels may be involved in the intrinsic mechanisms of myocardial protection during ischemia. We (Kamigaki et al., 1994) have reported that pretreatment of dogs with glibenclamide, a K_{ATP} channel blocker, worsens the myocardial metabolic derangement caused by ischemia. This drug increases insulin release through inhibition of pancreatic K_{ATP} channels, resulting in hypoglycemia. Worsening of ischemic myocardial damage by glibenclamide may be due to this hypoglycemia. The present study was, therefore, undertaken to examine the effect of glibenclamide on ischemic metabolic derangement of the heart with glucose infusion to abolish its hypoglycemic effect.

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2. Materials and methods

2.1. Animal preparations

49 mongrel dogs of either sex weighing 7–17 kg were anesthetized with pentobarbital sodium (30 mg/kg, i.v.), endotracheally intubated and ventilated with a respirator. A left thoracotomy was performed between the fourth and fifth ribs to expose the left ventricular wall. After suspending the heart in a pericardial cradle, the main trunk of the left anterior descending coronary artery was dissected free from the adjacent tissue to the first diagonal branch, and was loosely encircled with a silk thread for ligation. Ischemia was produced for 15 min by tightening this thread. Ischemia of a region of the myocardium was assessed by visible cyanosis and the elevation of the ST segment of the electrocardiogram (ECG) which was recorded from a wire electrode attached to the surface of the left ventricular wall. Aortic blood pressures were measured via a cannula introduced from the left femoral artery to near the aortic arch. Heart rate was counted from the ECG taken in the standard limb lead II. The double product was calculated from the systolic blood pressure and heart rate.

2.2. Experimental protocol

After about 30 min of control observations, the dogs were randomly divided into three groups: vehicle-treated, glibenclamide-treated, and glibenclamide + glucose-infused. Either vehicle (dimethyl sulfoxide, DMSO) or glibenclamide (1 mg/kg) was injected intravenously over a period of 30 s into the femoral vein in a volume of 0.3 ml/kg. Glucose (3 mg/kg per min) was continuously administered through a catheter into the femoral vein by means of a syringe-pump (Terumo Co., Tokyo) immediately after glibenclamide injection to the glibenclamide + glucose-infused group. Then, 10 min later, the ligature around the coronary artery was either not tied in the non-ischemic group or tied for 15 min in the 15-min ischemic group. After 15 min of ischemia, a transmural full-thickness sample was taken from the center of the ischemic region and frozen in liquid N_2 to measure the tissue metabolite levels. An equivalent sample was taken from the heart in the non-ischemic group 25 min after the injection.

2.3. Biochemical analysis

The frozen myocardium was pulverized in a mortar with a pestle precooled with liquid N_2 and extracted with 6% perchloric acid. The levels of glucose-6-phosphate, fructose-6-phosphate, fructose-1,6-diphosphate, lactate, ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP) and creatine phosphate in a neutralized perchloric acid extract were determined according to standard enzymatic procedures (Bergmeyer, 1974). The energy charge potential was calculated from the concentration of ATP, ADP and AMP to estimate the myocardial energy state according to the equation ([ATP] + 0.5[ADP])/([ATP] + [ADP] + [AMP]) (Atkinson and Walton, 1967). The ratio of ([glucose-6-phosphate] + [fructose-6-phosphate])/[fructose-1,6-diphosphate] was calculated from the concentration of hexose phosphates to estimate the rate of glycolytic flux through the phosphofructokinase reaction (Ichihara and Abiko, 1982). The blood glucose level was determined with a Reflolux II (Boehringer Mannheim-Yamanouchi, Co., Tokyo), and serum insulin was measured by radioimmunoassay (Yalow and Berson, 1959).

2.4. Statistical analysis

The data are expressed as means ± S.E. Hemodynamic data and the levels of blood glucose and insulin in the same dogs were evaluated by paired Student's *t*-test corrected for multiple comparisons by the Bonferroni method. Biochemical data and the double product between the groups were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. P values of 0.05 or less were considered significant.

3. Results

3.1. Serum insulin and blood glucose levels (Fig. 1)

The levels of serum insulin and blood glucose were not changed by vehicle injection. Glibenclamide in-

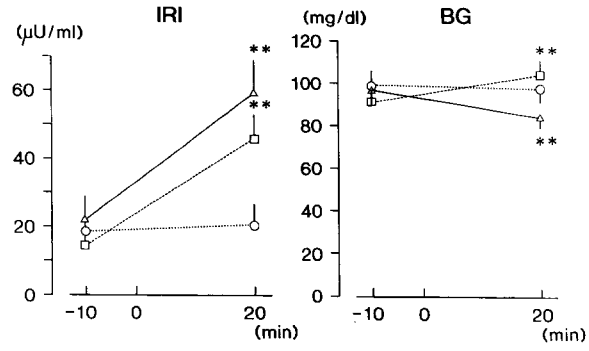

Fig. 1. Effects of glibenclamide with and without glucose infusion on serum insulin and blood glucose levels. Either vehicle (circles), or glibenclamide (triangles and squares) was injected at 0 min. Glucose (3 mg/kg per min) was continuously infused into the femoral vein immediately after the injection (squares). The left anterior descending coronary artery was ligated 10 min after the injection in the ischemic group. After 15 min, ischemia was terminated by removing the myocardium. Blood samples were taken 10 min before and 20 min after the injection. Data from non-ischemic and ischemic groups were combined. IRI = immunoreactive insulin, BG = blood glucose. $^{**}P < 0.01$, compared with the values obtained 10 min before the injection in each group.

Table 1
Changes in double products after glibenclamide injection and the onset of ischemia

	Vehicle-treated	Glibenclamide-treated	Glibenclamide-treated with glucose infusion
Non-ischemia	($n = 6$)	($n = 6$)	($n = 6$)
Before	25111 ± 2409	23083 ± 2038	24740 ± 2328
10 min	25681 ± 2726	20362 ± 1931	24375 ± 2439
25 min	27135 ± 3289	23415 ± 2171	24394 ± 1914
15-min ischemia	($n = 11$)	($n = 10$)	($n = 10$)
Before	24380 ± 2327	25960 ± 2223	21371 ± 1903
10 min	22441 ± 2253	25033 ± 1855	22368 ± 2178
25 min	23562 ± 2377	25979 ± 2021	22525 ± 2457

All data are the means (mm Hg/s) ± S.E.M. Glibenclamide (1 mg/kg) was injected i.v. 10 min before the onset of ischemia. The myocardium was removed 15 min after the onset of ischemia, namely, 25 min after the injection. Glucose infusion at 3 mg/kg per min was begun immediately after the glibenclamide injection. The number of animals is indicated in parentheses.

creased the serum insulin level to 270%, while it decreased the blood glucose level to 87%. Continuous infusion of glucose (3 mg/kg per min) abolished the hypoglycemia caused by glibenclamide, and increased the blood glucose level by 14% 20 min after the infusion. The serum level of insulin after glibenclamide injection with glucose infusion increased to the same extent to which it increased after glibenclamide injection alone.

3.2. Hemodynamic changes

Injection of vehicle did not change either arterial blood pressure or heart rate. Glibenclamide either without or with glucose infusion slightly increased blood pressure, while it decreased heart rate. No significant changes in blood pressure and heart rate were observed during ischemia in each group. Therefore, double products calculated from systolic blood pressure and heart rate were not significantly altered throughout the experiment in the vehicle-treated, glibenclamide-treated and glibenclamide + glucose-infused groups (Table 1).

3.3. Energy metabolism

The changes in the myocardial levels of energy metabolites are summarized in Table 2. In the vehicle-treated group, the levels of ATP, total adenine nucleotides, and creatine phosphate were significantly decreased by 15-min ischemia, while that of AMP was significantly increased. Injection of glibenclamide significantly enhanced the ischemia-induced decrease in ATP and total adenine nucleotides, regardless of glucose infusion. The calculated ECP is shown in Fig. 2. Ischemia significantly decreased ECP in all three groups. Glibenclamide alone appeared to enhance the decrease in ECP caused by ischemia, and glibenclamide + glucose infusion significantly worsened the ischemia-induced energy metabolism. Ischemia significantly decreased the level of creatine phosphate in all three groups. Although there was no significant differ-

Table 2
Changes in adenine nucleotides and creatine phosphate levels in non-ischemic and 15-min ischemic groups

Treatment	n	ATP	ADP	AMP	Total adenine nucleotides	Creatine phosphate
Non-ischemia						
Vehicle	6	5.06 ± 0.13	1.15 ± 0.05	0.18 ± 0.02	6.39 ± 0.17	4.62 ± 0.73
Glibenclamide	6	4.92 ± 0.13	1.01 ± 0.07	0.16 ± 0.02	6.09 ± 0.16	5.26 ± 0.79
Glibenclamide + glucose infusion	6	5.07 ± 0.20	1.06 ± 0.05	0.19 ± 0.01	6.32 ± 0.22	6.71 ± 0.58 [c]
15-min ischemia						
Vehicle	11	3.20 ± 0.21 [b]	1.11 ± 0.05	0.23 ± 0.01 [a]	4.54 ± 0.22 [b]	2.13 ± 0.32 [b]
Glibenclamide	10	2.42 ± 0.15 [b,d]	1.00 ± 0.03	0.22 ± 0.01 [b]	3.64 ± 0.16 [b,d]	1.65 ± 0.22 [b]
Glibenclamide + glucose infusion	10	2.36 ± 0.17 [b,d]	1.04 ± 0.04	0.25 ± 0.01 [b]	3.65 ± 0.20 [b,d]	1.75 ± 0.23 [b]

Data are the means ± S.E.M. and are expressed as μmol/g wet tissue except for total adenine nucleotides. n = number of animals. [a] $P < 0.05$; [b] $P < 0.01$, compared with non-ischemia in each group. [c] $P < 0.05$; [d] $P < 0.01$, compared with the vehicle-treated non-ischemic and ischemic group.

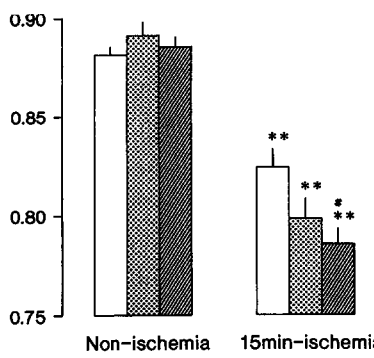

Fig. 2. Effects of glibenclamide with and without glucose infusion on ECP. The energy charge potential (ECP) was calculated according to the equation ([ATP]+0.5[ADP])/ ([ATP]+[ADP]+[AMP]). Open columns, stippled columns and hatched columns represent vehicle-treated, glibenclamide-treated and glibenclamide + glucose infusion groups, respectively. The myocardial tissue samples were taken 15 min after the onset of ischemia (15-min ischemia). An equivalent sample was taken from the heart whose coronary artery was not ligated 25 min after the injection (non-ischemia). $^{**}P < 0.01$, compared with non-ischemia in each group. $^{\#}P < 0.05$, compared with the vehicle-treated ischemic group.

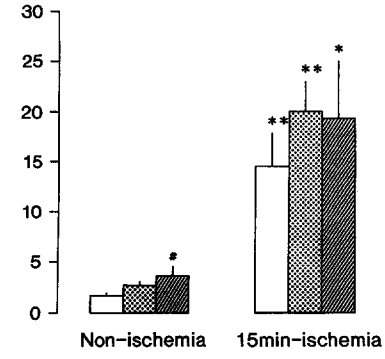

Fig. 3. Effects of glibenclamide with and without glucose infusion on ([glucose-6-phosphate] + [fructose-6-phosphate])/[fructose-1,6-diphosphate] ratio. Symbols are the same as those in Fig. 2. $^{*}P < 0.05$; $^{**}P < 0.01$, compared with non-ischemia in each group. $^{\#}P < 0.05$, compared with the vehicle-treated animals in non-ischemic and 15-min ischemic groups.

ence among the groups, the decrease in creatine phosphate level due to ischemia tended to be enhanced by pretreatment with glibenclamide even when glucose was infused continuously.

3.4. Carbohydrate metabolism

Changes in the hexose phosphate levels, and the lactate level due to ischemia are summarized in Table 3. Ischemia significantly increased the levels of glucose-6-phosphate and fructose-6-phosphate, whereas it appeared to decrease the level of fructose-1,6-diphosphate. Therefore, the ratio ([glucose-6-phosphate] + [fructose-6-phosphate])/[fructose-1,6-diphosphate] increased significantly during ischemia, indicating inhibition of the glycolytic flux at the level of the phosphofructokinase reaction (Fig. 3). Although there was no statistical significance, pretreatment with glibenclamide tended to enhance the ischemia-induced accumulation of glucose-6-phosphate and fructose-6-phosphate and reduction of fructose-1,6-diphosphate, resulting in augmentation of the ratio of ([glucose-6-phosphate] + [fructose-6-phosphate])/[fructose-1,6-diphosphate] (Fig. 3). Similar results were obtained in the glibenclamide + glucose-infused group. Ischemia significantly increased the lactate level in all three groups. Glibenclamide significantly augmented the ischemia-induced increase of lactate level as compared with vehicle. Glucose infusion attenuated the augmentation of ischemia-induced lactate accumulation caused by glibenclamide. However, the lactate level still appeared to be higher than that in the vehicle-treated ischemic myocardium.

4. Discussion

In the present study, continuous infusion of glucose maintained or slightly increased the blood glucose level even after glibenclamide injection (Fig. 1). Although glucose was infused, the increased level of serum in-

Table 3
Changes in hexose phosphate levels in non-ischemic and 15-min ischemic groups

Treatment	n	Glucose-6-phosphate	Fructose-6 phosphate	Fructose-1,6-diphosphate	Lactate
Non-ischemia					
Vehicle	6	0.20 ± 0.03	0.04 ± 0.02	0.14 ± 0.02	1.51 ± 0.14
Glibenclamide	6	0.25 ± 0.05	0.06 ± 0.02	0.13 ± 0.03	1.70 ± 0.30
Glibenclamide + glucose infusion	6	0.21 ± 0.04	0.04 ± 0.01	0.08 ± 0.01 [c]	1.32 ± 0.21
15-min ischemia					
Vehicle	11	0.92 ± 0.20 [b]	0.21 ± 0.06 [a]	0.09 ± 0.02	14.91 ± 2.35 [b]
Glibenclamide	10	1.40 ± 0.23 [b]	0.35 ± 0.06 [b]	0.09 ± 0.01	23.32 ± 2.37 [b,c]
Glibenclamide + glucose infusion	10	1.05 ± 0.21 [b]	0.28 ± 0.06 [b]	0.09 ± 0.02	17.93 ± 1.94 [b]

Data are the means ± S.E.M. and are expressed as μmol/g wet tissue. n = number of animals. [a] $P < 0.05$; [b] $P < 0.01$, compared with non-ischemia in each group. [c] $P < 0.05$, compared with the vehicle-treated non-ischemic and ischemic group.

sulin due to glibenclamide was similar to the level obtained without glucose infusion. Because the blood glucose level after 3 mg/kg per min of glucose infusion was still within the physiological ranges, the serum level of insulin did not increase further. In spite of cancellation of hypoglycemia, glibenclamide enhanced the ischemia-induced myocardial metabolic derangement, and enhanced the myocardial high-energy depletion and the accumulation of hexose phosphates and lactate (Tables 2 and 3). However, there were no significant differences in high-energy phosphates and hexose phosphates between the glibenclamide-treated and the glibenclamide + glucose-infused groups. Double products, an indicator of cardiac oxygen consumption, were not changed by glibenclamide injection either with or without glucose infusion. These findings suggest that the deleterious effect of glibenclamide on the ischemic myocardial metabolism is not due to its hypoglycemic effect. The present results strongly support the role of K_{ATP} channels in endogenous mechanisms which serve a cardioprotective function (Gross and Auchampach, 1992; Cole, 1993)

Previous in vitro studies using the electrophysiological patch-clamp technique with cardiac myocytes have shown that K_{ATP} channels begin to open as the intracellular concentration of ATP decreases by approximately less than 0.5 mM (Noma, 1983; Deutsch et al., 1991), whereas in an in vivo system like our experiment, the concentration of the tissue ATP level is still around 3 mM even after 15-min ischemia. Accordingly, the K_{ATP} channels of the ischemic myocardium in vivo still seem to be closing. However, the open probability of K_{ATP} channels appears to be increased not only by the decrease of tissue ATP level but also by other modulators, i.e., the decrease of the ATP/ADP ratio (Nichols and Lederer, 1991), intracellular ATP compartmentation (Gudbjarnason et al., 1970), acidosis (Cuevas et al., 1991), and the activation of G protein through adenosine A_1 receptors (Kirsh et al., 1990). Since a K_{ATP} channel has high single-channel conductance and a very high membrane density, the opening of a remarkably small number of K_{ATP} channel is able to influence the duration of the cardiac action potential (Faivre and Findlay, 1990; Cavero and Guillon, 1993). The multiplied interaction of these factors would effectively promote K_{ATP} channel opening in spite of a high ATP level.

K_{ATP} channels of cardiac muscle are less susceptible to sulfonylureas than are those of pancreatic β-cells. There is a 10-fold difference in sensitivity between them (Fosset et al., 1988). This leads us to consider that clinical use of sulfonylureas may not present any problem with the ischemic myocardium. However, ischemia produces marked acidification in both the intracellular and the extracellular environment (Ichihara et al., 1979). According to the report by Findlay (1992), extracellular acidification causes a marked increase in the concentration of the unionized forms of sulfonylurea, and this form is responsible for closing of K_{ATP} channels in cardiac muscle. Therefore, he (Findlay, 1992) suggests that extracellular acidification during ischemia will increase the effective concentration of glibenclamide to the extent that it cancels the difference in sensitivity between the tissues and may be responsible for a high incidence of cardiovascular disorders in diabetic patients treated with sulfonylureas.

In conclusion, glibenclamide enhances the ischemic myocardial metabolic derangement, and myocardial K_{ATP} channels may play a role as an endogenous mechanism to protect the myocardium against further ischemic injury.

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