Adenosine is Required for Myocardial Insulin Responsiveness In Vivo

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The adenosine-receptor antagonist 8-phenyltheophylline (8-PTH) was used to study the role of endogenous adenosine in modulating insulin-stimulated myocardial glucose uptake (MGU) in vivo. Dogs were surgically instrumented under pentobarbital sodium anesthesia to measure hemodynamics and obtain blood samples for determinations of oxygen and glucose concentrations. Myocardial uptake of these substances was calculated as the product of the appropriate arterial–coronary sinus differences and circumflex blood flow. The response to insulin was determined with the hyperinsulinemic-euglycemic clamp technique. During insulin infusion, MGU increased from 3.12 ± 0.8 to 9.20 ± 1.8 mg/min (mean ± SE). In contrast, insulin failed to increase MGU when 8-PTH was being infused into the circumflex artery. These results demonstrate that some degree of adenosine-receptor–mediated activity is required for insulin to stimulate myocardial glucose uptake. It is suggested that the presence of adenosine at its receptor may be an important factor during conditions in which myocardial insulin resistance may develop. *Diabetes* 37:842–45, 1988

We recently reported that adenosine potentiated insulin-stimulated glucose uptake in the heart in vivo (1). In this respect the heart was shown to respond in a manner similar to adipose tissue, where adenosine also increases responsiveness to insulin (2,3). Joost and Steinfelder (4) have reported that theophylline can block insulin's ability to stimulate adipocyte glucose uptake. Because theophylline has significant adenosine-receptor–antagonist activity (5) and the heart responds to adenosine in a manner similar to adipose tissue, we hypothesized that adenosine-receptor blockade in the heart would prevent insulin's action on glucose transport therein. To test this hypothesis, we used 8-phenyltheophylline (8-PTH), a compound with more adenosine-receptor–antagonist activity and less phosphodiesterase-inhibiting activity than theophylline (5,6). Thus, our study was undertaken to determine if adenosine-receptor–mediated activity is required for insulin stimulation of myocardial glucose uptake in vivo.

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Received for publication 7 January 1988 and accepted in revised form 1 March 1988.

MATERIALS AND METHODS

Twenty-one conditioned mongrel dogs of either sex weighing 20–25 kg were used in this study. Dogs were anesthetized with pentobarbital sodium (35 mg/kg i.v.), intubated, and ventilated with room air against 3–5 cmH_2O-positive end-expiratory pressure by use of a Harvard respirator. Supplemental doses of pentobarbital sodium were administered intravenously as needed. After administering succinylcholine (1 mg/kg i.v.), a left thoracotomy was performed in the 5th intercostal space. The pericardium was longitudinally transected, and the heart was exposed. A silastic cannula was placed into the coronary sinus via the right atrial appendage and was used to obtain venous blood samples. A Gould electromagnetic flow probe was placed around the circumflex artery to measure coronary blood flow with a Gould SP 2202 flowmeter. A 1-inch 22-gauge angiocatheter was inserted anterograde into the circumflex artery for infusion of saline, 8-PTH, or the vehicle for 8-PTH at a rate of 1 ml/min. Saline was infused into the circumflex artery to maintain catheter patency whenever other agents were not being administered by this route and to act as a volume control. Circumflex blood flow was not affected by placement of the angiocatheter.

A femoral arterial cannula was advanced into the abdominal aorta to measure arterial blood pressure and obtain arterial blood samples. Two femoral venous cannulas were advanced into the inferior vena cava and used to infuse

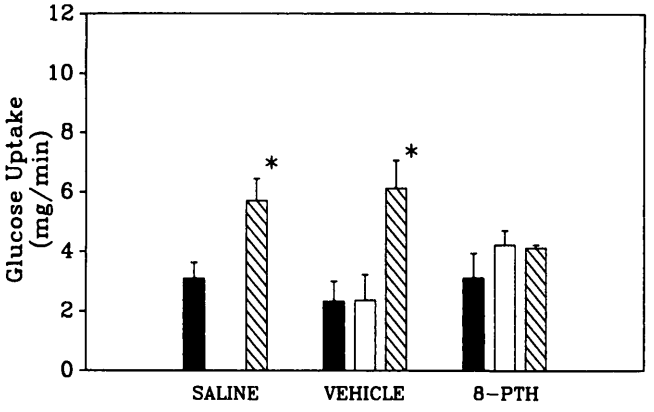

FIG. 1. Myocardial glucose uptake during saline basal period (*solid bars*), control infusion of vehicle or 8-phenyltheophylline (*open bars*), and after establishment of hyperinsulinemic-euglycemic clamp (*hatched bars*). Values are means ± SE. *$P < .05$ vs. basal value.

insulin and glucose. On-line hemodynamic measurements were recorded on a Gould 2800s physiological recorder. Arterial and coronary sinus blood gases, pH, and oxygen content were determined with a radiometer Acid-Base Laboratory (ABL-3). Blood glucose was determined with a Yellow Springs 23A glucose analyzer. Myocardial uptake of glucose and oxygen were calculated as the product of the appropriate arterial–coronary sinus concentration difference and circumflex blood flow.

After surgical instrumentation, animals were allowed to stabilize for at least 60 min. Respirator volumes and rates were set by careful monitoring of arterial blood gases and pH status before initiating the temporal protocol.

Basal hemodynamic and metabolic profiles were determined during intracoronary infusion of saline. Dogs then received 8-PTH (10 μmol/min, $n = 5$), the vehicle for 8-PTH ($n = 5$), or a continuation of intracoronary saline infusion ($n = 7$) for 20 min. The effect of 8-PTH or its vehicle on hemodynamic and metabolic parameters was then determined (control values in figures and tables). Thereafter, the response to insulin was determined with the hyperinsulinemic-euglycemic clamp technique while intracoronary 8-PTH, vehicle, or saline infusion continued. In a separate group of dogs ($n = 4$) the response to insulin was followed over 3 h with intracoronary saline infusion only. Regular insulin was administered as a bolus (1.5 U/kg) followed by constant infusion (4 U/min) at a rate of 1 ml/min. A 20% D-glucose solution was infused at variable rates to maintain blood glucose at basal values. The euglycemic clamp was accepted when the arterial glucose concentration was within 5 mg/dl of the basal arterial concentration for three successive measurements made 5 min apart at a constant glucose infusion rate. Insulin was infused for a minimum of 60 min before measuring the responses to insulin. At the completion of the experiments, the dogs were killed by the rapid intravenous injection of concentrated potassium chloride.

Adenosine and 8-PTH were obtained from Calbiochem (San Diego, CA). Regular insulin was obtained from Lilly (Indianapolis, IN). A solution of 0.9% saline containing 2.5% 1 M NaOH and 7.5% ethanol was used as the vehicle to dissolve the 8-PTH to a final concentration of 10^{-2} M.

Data were analyzed via Bartlett's test for homogeneity of variances, followed by an analysis of variance for repeated measures. Subsequent analyses were by Duncan's multiple-range test with α set at .05 for the differences among means.

RESULTS

The changes in myocardial glucose uptake are displayed in Fig. 1. Myocardial glucose uptake was significantly elevated during clamp when either saline or the 8-PTH vehicle was infused. In contrast to this, myocardial glucose uptake did not change in response to insulin during 8-PTH infusion. Neither vehicle nor 8-PTH had any effect on basal glucose uptake. When insulin infusion and clamp were maintained for 3 h, myocardial glucose uptake did not change significantly (Fig. 2), indicating that the response to a 4-U/min dose of insulin reached a maximal value by 1 h.

The completeness of adenosine blockade was tested by observing the vasodilatory response to a pulse of 0.3 ml of 10^{-4} M adenosine through the circumflex infusion catheter before and during 8-PTH infusion. During 8-PTH infusion, the blood flow response to adenosine was severely attenuated (data not shown).

Changes in hemodynamic parameters, myocardial oxygen uptake (MVO_2), and the arterial–coronary sinus difference for glucose are listed in Table 1. Circumflex blood flow and the arterial–coronary sinus difference for glucose were elevated by clamp during infusion of saline or vehicle. These changes were not observed during 8-PTH infusion. No significant changes were observed in the other parameters listed.

In animals that received intracoronary saline, plasma insulin levels rose significantly from 24.36 ± 6.8 to 37,120 ± 3578 μU/ml (means ± SE) during the clamp. Infusion of 8-PTH alone had no significant effect on plasma insulin compared with basal levels (16.8 ± 4.6 vs. 26.5 ± 8.4 μU/ml, respectively). The clamp resulted in significantly elevated plasma insulin values of 48,650 ± 1294 μU/ml during 8-PTH infusion.

DISCUSSION

These data demonstrate that adenosine-receptor–mediated activity in the myocardium in vivo is required for insulin to

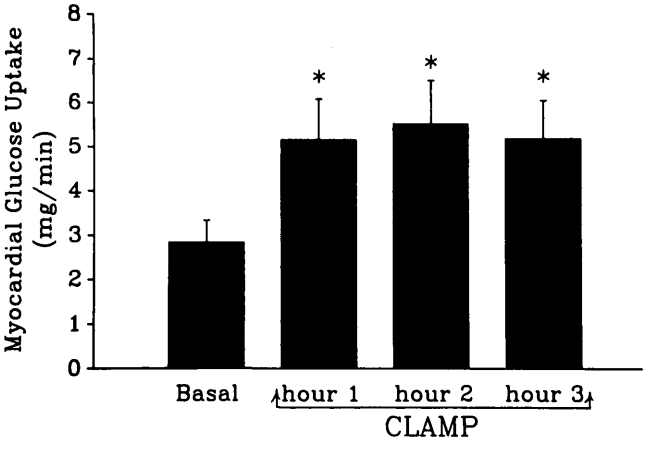

FIG. 2. Myocardial glucose uptake during saline basal period and at 1, 2, and 3 h after establishment of hyperinsulinemic-euglycemic clamp. Values are means ± SE. *$P < .05$ vs. basal value.

TABLE 1
Changes in hemodynamic parameters, MVO_2, and the arterial–coronary sinus difference (A–CS) for glucose during infusions under basal, control, and clamp conditions

	Saline (n = 7)		Vehicle (n = 5)			8-Phenyltheophylline (n = 5)		
	Basal	Clamp	Basal	Control	Clamp	Basal	Control	Clamp
Mean arterial pressure (mmHg)	110 ± 8.5	110.8 ± 5.0	116.3 ± 7.9	116 ± 13.2	120 ± 13.6	110 ± 8.5	114.6 ± 4.0	106 ± 5.8
Blood flow (ml/min)	58 ± 4.6	82.8 ± 19.2*	50.4 ± 5.4	63.8 ± 7.4	82.8 ± 10.1*	58 ± 4.5	67.2 ± 3.8	79.4 ± 11.5
Heart rate (beats/min)	148 ± 3.0	156 ± 5.8	154 ± 5.4	138.7 ± 9.4	140.3 ± 13.1	143 ± 5.6	151.4 ± 4.3	152.6 ± 8.2
MVO_2 (ml/min)	5.57 ± 0.7	7.67 ± 1.2	4.63 ± 0.7	5.14 ± 0.7	6.09 ± 0.8	5.56 ± 0.7	7.46 ± 0.4	7.38 ± 1.1
A–CS glucose (mg/dl)	5.38 ± 1.7	11.38 ± 1.1*	4.5 ± 1.3	4.0 ± 1.6	8.5 ± 0.6*	5.3 ± 1.5	6.5 ± .89	6.13 ± 1.9

*$P < .05$, significantly different from basal value.

stimulate glucose uptake. When adenosine receptors were blocked by 8-PTH, a competitive receptor antagonist, insulin was unable to increase myocardial glucose uptake.

We have previously reported that exogenously administered adenosine in vivo potentiated insulin-stimulated myocardial glucose uptake in a dose-dependent manner (1). We demonstrated that this effect occurred independent of changes in coronary blood flow, MVO_2, and lactate uptake and was specific for this nucleoside (1). However, because we had used exogenously administered adenosine, the influence of endogenous adenosine levels on insulin's actions had not been addressed. In this study, we used 8-PTH, an alkylxanthine derivative that has been shown to provide 100% adenosine-receptor blockade with only minimal effects on phosphodiesterase activity at the concentration that we used (5,6). The effectiveness of this blockade was confirmed during our experiments by observing the loss of a vasodilatory response to adenosine infused into the circumflex artery during 8-PTH infusion. The fact that insulin was unable to increase myocardial glucose uptake in the presence of this adenosine-receptor–blocking agent demonstrates that the presence of adenosine in the concentrations produced under physiological conditions is required for this effect of insulin in heart and that adenosine's ability to allow myocardial insulin responsiveness is an adenosine-receptor–mediated phenomenon.

The myocardial response to insulin alone that we report herein is quantitatively similar to that reported by Downing and Lee (8) in their work with neonatal lambs. Their studies indicate that the maximal myocardial response to insulin occurs before 60 min when administered as a bolus. We used a bolus followed by a constant infusion of insulin. Establishment of hyperinsulinemic-euglycemic clamp required 45–60 min, suggesting that at the dose of insulin that we used, the response is already maximized by the first clamp measurement. In support of this, we found that myocardial glucose uptake remained constant when the clamp was maintained for 3 h (Fig. 2).

The blockade of insulin-stimulated myocardial glucose uptake by 8-PTH cannot be attributed to hemodynamic alterations or changes in MVO_2. There were no significant differences in blood pressure, heart rate, or MVO_2 between the groups. Although 8-PTH prevented insulin-induced increases in blood flow, its primary effect was manifested in its ability to prevent the increased arterial–coronary sinus difference for glucose that insulin normally causes. Therefore, the ability of 8-PTH to block insulin's effect on myocardial glucose uptake is not a flow-dependent phenomenon, which agrees with our previous findings (1).

We previously reported that this response of the myocardium to adenosine is similar to that of adipose tissue but opposite to skeletal muscle (1), where adenosine attenuates the action of insulin (9,10). There is also some evidence that suggests that adenosine is required for insulin's action in adipose tissue. Green (11) has reported that adipocytes in which adenosine receptors are downregulated demonstrate reduced responsiveness to insulin. Joost and Steinfelder (4) have reported that 2.5-mM concentrations of theophylline can block the stimulatory action of as much as 6 ng/ml insulin, reducing in vitro adipocyte 2-deoxyglucose uptake to zero. They suggest that this occurs as a result of the lipolytic activity of theophylline. An alternate explanation is that the blockade of insulin-stimulated glucose uptake that they report occurs as a result of the adenosine-receptor–antagonist activity of theophylline (5). We obtained similar results in this study in the heart in vivo by use of 8-PTH, which is more specific as an adenosine-receptor antagonist (5) and is relatively inactive as a phosphodiesterase inhibitor (5,6). It is therefore apparent that some endogenous level of adenosine, acting at its receptor, is required for insulin action in the heart.

Before this study and our previous report, little had been done to examine the specific interaction between adenosine and insulin with respect to myocardial glucose uptake. Results from a Langendorff perfused-heart experiment performed by Fuller and Sugden (7) appear to disagree with our findings in vivo (1). Although the reason for this difference remains unclear, it is probably related to difficulties inherent in extrapolating in vitro data to an in vivo environment. These potential problems have been discussed in detail (1).

In summary, our data suggest that the presence of adenosine in myocardial tissue is required for insulin to stimulate glucose uptake and that this effect of adenosine occurs via a receptor-mediated event. Studies are underway to further characterize and define this receptor-mediated phenomenon.

ACKNOWLEDGMENTS

This study was supported by NIH Grant HL-31163 and the Veterans Administration.

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