Adenosine effects on glucose oxidation of adipocytes isolated from streptozotocin-diabetic rats

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Streptozotocin-induced diabetes did not impair the response of adipocytes to adenosine effects in glucose oxidation. The greatest effect of adenosine in potentiating the action of insulin was in the physiological concentration range of insulin (10–100 μ units/ml). The desensitization of cells by diabetes to the effects of insulin is therefore probably not related to the response of cells to adenosine.

INTRODUCTION

Receptors for adenosine in plasma membranes of adipocytes have been identified and characterized [1]. This has lent further credence to the belief that the role of adenosine as an endogenous regulator of adipocyte metabolism could be one of significance. The nucleoside has been shown to be released spontaneously by isolated adipocytes into the incubation medium and to modulate the metabolic activity of the cells [2, 3]. In its presence, hormone-induced lipolysis and cyclic AMP accumulation were inhibited. Adenosine can mimic insulin effects on glucose transport and oxidation [4, 5], cyclic AMP accumulation and phosphodiesterase activity [6–11], lipolysis and lipid synthesis [4, 5], pyruvate dehydrogenase activity [12, 13] and leucine oxidation [12]. The sensitivity of the fat-cell to insulin activation of glucose transport and oxidation and pyruvate dehydrogenase activity is also enhanced by adenosine [5, 4, 13]. It has been shown that catecholamines completely inhibited insulin-stimulated 2-deoxyglucose uptake by rat adipocytes, but only when adenosine was prevented from accumulating in the incubation medium by the addition of adenosine deaminase [14]. Since there appears to be a strong inter-relationship between the effects of adenosine and those of insulin, it was decided to investigate the effects of adenosine on adipocytes from diabetic animals.

The present study compares the effects of adenosine on glucose oxidation and pyruvate dehydrogenase activity of adipocytes isolated from normal and streptozotocin-diabetic rats. The relationship of these effects to those of insulin was also examined. The adenosine analogue PIA, which binds to adenosine receptors but is not metabolized by adenosine deaminase, was used to study the adenosine effects. This analogue has been shown to have the same effects as adenosine on fat-cells [13].

MATERIALS AND METHODS

Insulin was obtained from Dr. R. Chance, Eli Lilly Co. (Indianapolis, IN, U.S.A.), adenosine deaminase from Sigma, PIA from Boehringer, and [$1\text{-}^{14}\text{C}$]pyruvic acid and [$1\text{-}^{14}\text{C}$]glucose were from New England Nuclear (Boston, MA, U.S.A.). All other chemicals and biochemicals were from standard sources.

Diabetes was induced by intramuscular injection of streptozotocin (60 mg/kg) in citrate buffer, pH 4.5, by the method of Nakhoda & Wong [15]. The animals were maintained for 7 days before use. Blood glucose, determined with a glucose kit from Sigma, was always greater than 250 mg/dl.

Isolated fat-cells were prepared by the collagenase method of Rodbell [16] from the epididymal adipose tissues of fed male Sprague–Dawley rats (150–170 g). Krebs–Ringer bicarbonate buffer [17], pH 7.4, containing 2.5 mM- Ca^{2+} and 4% (w/v) bovine serum albumin was used. Cells were prepared in the presence of adenosine deaminase (2 units/ml) to minimize the accumulation of endogenous adenosine and its effects. Cells so prepared, apart from having a more consistent response to adenosine, did not differ from those prepared in the absence of adenosine deaminase [11]. For glucose-oxidation studies, the fat-cells were washed in Krebs–Ringer phosphate buffer, pH 7.4, before use. The study was carried out as described by Glieman [18].

The experiments on pyruvate dehydrogenase activity of intact adipocytes were carried out as described previously [13]. Protein measurements were performed on samples of adipocytes from both normal and diabetic animals.

RESULTS AND DISCUSSION

Effects of increasing concentrations of insulin on glucose oxidation in the presence and absence of PIA

The sensitivity of adipocytes to insulin activation of glucose transport and oxidation has been shown to be enhanced by adenosine [4, 5, 10, 12]. Whether this phenomenon was affected by diabetes was investigated. For comparison, the effects of increasing concentrations of insulin on the oxidation of glucose were studied with adipocytes isolated from both normal (Fig. 1a) and streptozotocin-diabetic rats (Fig. 1b) in the presence and absence of adenosine. Adenosine deaminase, with or without the non-metabolizable adenosine analogue PIA, was used, and 1 μM -PIA was the chosen concentration because maximal stimulation of glucose oxidation was obtained with 0.1–1.0 μM (Figs. 2 and 3).

Adipocytes from normal rats metabolized glucose at a

Abbreviations used: PIA, N^6 -phenylisopropyladenosine.

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Fig. 1. Effect of insulin concentration on glucose oxidation in the presence of PIA

Adipocytes were isolated from normal (a) and diabetic (b) rats in the absence (○) or presence (●) of PIA. Fat-cells were preincubated for 15 min before determination of glucose oxidation for 60 min as described by Glieman [18]. Experiments were carried out in the presence of 1 unit of adenosine deaminase/ml. Results of a typical experiment performed in triplicate are shown. Bar above each data point shows the s.d.

faster rate than did those from diabetic animals, and glucose oxidation was maximally stimulated by 100 μ units of insulin/ml in the presence of adenosine and by 1 munit of insulin/ml in the absence of adenosine (Fig. 1). Cells from diabetic animals were, on the other hand, less sensitive to insulin, and required a higher insulin concentration to be maximally stimulated. In both types of cells, however, adenosine increased the sensitivity of the adipocyte to the effects of insulin. In cells from diabetic animals, adenosine not only caused a leftward shift in the dose-response curve to insulin, as observed for normal adipocytes, but also potentiated greatly the maximal effect of insulin alone. PIA (1 μ M) enhanced the effect of 100 μ units of insulin/ml to beyond that of 10 munits of insulin/ml alone (Fig. 1b). The greatest effect of adenosine appears to be in the physiological concentration range of insulin, 10–100 μ units/ml, especially the lower range or starvation value (10 μ units/ml) of insulin. At this concentration of insulin, without adenosine, there would have been little or no glucose oxidation above basal values. From the above experiments, it could be concluded that the impairment of the sensitivity of adipocytes to insulin in diabetic animals was not due to a breakdown of the mechanism by which adenosine potentiates the effects of insulin. Thus the role of adenosine appears to be to sensitize the fat-cell to insulin, in particular the effects of low concentrations of insulin, as during starvation or when the sensitivity of adipocytes to insulin is decreased as a result of diabetes.

Effects of increasing concentrations of PIA on glucose oxidation in the presence of suboptimal and optimal insulin concentrations

Diabetes is known to cause a desensitization of adipocytes to insulin. Whether this was also the case with adenosine was investigated by comparing the effects of various concentrations of adenosine. Adenosine effects

were also studied in the presence of physiological concentrations of insulin (suboptimal 10 μ units/ml and optimal 100 μ units/ml).

Results presented in Fig. 2 show that, although the rate of glucose oxidation in adipocytes was depressed by diabetes, the concentration of adenosine required to stimulate the basal rate by 100% in adipocytes from both normal and diabetic rats was the same (0.1 μ M). Maximal stimulation was obtained with 0.1–1 μ M-adenosine in both types of adipocytes. This was not so when the cells were stimulated with insulin. Cells from normal rats responded to a greater degree (8-fold) than did cells from diabetic rats (5-fold), and a greater insulin concentration was required by the latter (10 munits/ml) than by the former (1 munit/ml) to reach maximal stimulation (Figs. 1a and 1b respectively).

Fig. 2 also shows that the addition of a small amount of insulin (10 μ units/ml), which in itself had little or no effect, enhanced the adenosine effect in normal adipocytes by more than 100% but that on diabetic cells by only 50%. This difference is likely to be the result of a decreased sensitivity to insulin, since the sensitivity of the cells to adenosine was not affected by diabetes. Thus the present study shows that diabetes impairs the response of cells to insulin, but not to adenosine.

It was noteworthy that adenosine in increasing concentrations caused a biphasic effect on glucose oxidation in adipocytes from both normal and diabetic rats. PIA at 100 μ M had no effect or was slightly inhibitory. This biphasic effect of adenosine has also been observed in other aspects of fat-cell metabolism [19, 20]. Pyruvate dehydrogenase was stimulated in a similar manner [13], and so were the incorporations of glucose and fructose into glycogen [21]. The cause of this phenomenon is at present unknown. The production of inhibitors in response to high concentrations of adenosine has yet to be established.

Fig. 2. Effect of suboptimal insulin dose and increasing PIA concentrations on glucose oxidation by adipocytes

Experimental conditions were similar to those of Fig. 1, except that incubations were carried out in the absence (○) or presence (●) of 10 μ units of insulin/ml.

Fig. 3. Effect of optimal insulin dose and increasing PIA concentrations on glucose oxidation by adipocytes

Experimental conditions were similar to those of Fig. 1, except that incubations were carried out in the absence (○) or presence (●) of 100 μ units of insulin/ml.

The enhancing effects of insulin also diminished with high adenosine concentrations (Figs. 2 and 3). Fig. 3 shows that 100 μ M-adenosine in fact decreased the effects produced by 100 μ units of insulin/ml alone. Combinations of optimal concentrations of insulin and adenosine have also been observed under certain conditions to be less effective than was insulin alone (E. H. A. Wong & S. O. Ooi, unpublished work). Whether this is indicative of an interaction between the mechanism(s) of action of

adenosine and that of insulin requires further investigation.

Effects of insulin and PIA on pyruvate dehydrogenase activity of adipocytes from normal and diabetic rats

Pyruvate dehydrogenase of adipocytes, an insulin-sensitive enzyme, has been shown to be stimulated by adenosine [13]. The mechanism by which adenosine affected this enzyme appears to be related to that of

Table 1. Effect of insulin and PIA on pyruvate dehydrogenase activity of adipocytes from normal and diabetic rats

Adipocytes were isolated from normal and diabetic adipose tissue as indicated in the Materials and methods section. Cells were incubated in Krebs–Ringer bicarbonate buffer, pH 7.4, for 10 min in the presence of adenosine deaminase (1 unit/ml) and the agents indicated in the Table. Pyruvate dehydrogenase activity was determined as previously described [13]. Results are means $\pm$ S.E.M. ($n = 6$): ** $P < 0.01$, * $P < 0.05$ versus appropriate control.

	PDH activity (pmol/min per 10 mg of protein)
Expt. A: normal	
Basal	384 $\pm$ 14
PIA (100 μ M)	675 $\pm$ 13**
Insulin (100 μ units/ml)	747 $\pm$ 13**
Expt. B: diabetic	
Basal	60.0 $\pm$ 3.5
PIA (1 μ M)	61.6 $\pm$ 3.0
PIA (10 μ M)	77.8 $\pm$ 2.8*
PIA (50 μ M)	67.0 $\pm$ 1.8
PIA (100 μ M)	63.3 $\pm$ 1.7
Insulin (100 μ units/ml)	78.3 $\pm$ 3.3*

insulin. Hence it would be interesting to see whether diabetes altered the effects of adenosine on this enzyme.

Table 1 shows the effects of insulin and PIA on pyruvate dehydrogenase activity of adipocytes isolated from normal and diabetic rats. Since the dose–response of cells from normal animals to adenosine had been previously studied [13], experiments were carried out only with the optimal concentrations of insulin and PIA for comparison purposes. PIA at 100 μ M had an effect which was almost equal to that of 100 μ units of insulin/ml. The response of cells to PIA in this series of experiments was particularly good. Whether this was due to the efficacy of the adenosine deaminase preparation to remove exogenous adenosine could not be confirmed.

The pyruvate dehydrogenase activity of cells from diabetic animals was low and responded poorly to both insulin and adenosine (PIA) stimulation. There was, however, a difference between the effects of adenosine and of insulin. For maximal stimulation of the enzyme, cells from diabetic animals required a lower PIA concentration than did cells from normal animals (Table 1), whereas with the insulin the optimal concentration for the former cells was similar if not greater than that required for the latter cells (results not shown). Whether this difference is related to the dual stimulatory and inhibitory effects of adenosine requires further investigation. The phenomenon of diabetic cells being more sensitive to suboptimal doses of hormones, resulting in a leftward shift in their dose–response curve to hormones, has also been previously observed [21].

The high PIA concentrations required to stimulate

pyruvate dehydrogenase activity, a mitochondrial enzyme, could be a result of penetration problems. It could also be indicative of the lesser role of adenosine on insulin action on this enzyme than on glucose oxidation as a whole.

In conclusion, the present study provides further evidence that the role of adenosine in fat-cell metabolism could be closely related to that of insulin. This could be important particularly at low insulin concentrations and when the normal response to insulin is decreased because of diabetes. Although diabetes depressed the rate of glucose oxidation and decreased pyruvate dehydrogenase activity, the role of adenosine, in sensitizing the fat-cell to insulin, was not affected. Since this could be the major role of adenosine, the elucidation of the mechanism of adenosine action may provide some insight into the understanding of insulin action and the diabetic state.

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