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In vitro insulin action on erythrocyte glucose metabolism in normal and diabetic rats

V. R. Agarwal, A. K. Rastogi and P. Sagar

Division of Biochemistry, Central Drug Research Institute, Lucknow, India

Summary. Alloxan-induced diabetes in rats significantly impaired the capacity of the erythrocytes to metabolise glucose in vitro to either lactic acid or CO_2. Both these metabolic activities were initially insensitive to insulin in normal as well as in diabetic animals; but became responsive when these cells were subjected to insulin and glucose 'starvation' for 1 h through incubation in their absence. This action of insulin in starved cells showed concentration dependence and required preincubation with the hormone prior to addition of glucose.

Key words: Insulin, erythrocyte, diabetes, glucose metabolism, rat.

Whereas erythrocytes have generally been considered insensitive to insulin in the past, Gambhir et al. [1] demonstrated the presence of insulin receptors on these cells, whose characteristics resembled the properties of hormone receptors of classical target tissues e.g. liver, fat and muscle cells. Further, we have observed changes in the $Na^+ + K^+$ and Ca^{2+}-ATPases [2] and acetylcholinesterase [3] in erythrocyte ghosts on in vitro incubation with the hormone. Baldini et al. [4] recently reported that contrary to an inhibitory response of insulin towards ghost $Na^+ + K^+$-ATPase, exposure of intact erythrocytes to the hormone may stimulate its Na-pumping mechanism. A similar response of insulin was noted earlier in frog skeletal muscle, which Moore [5] considered to be indirectly responsible for some of the well-known physiological responses of the hormone, including the modulation of glycolysis through an indirect influence on phosphofructokinase.

The erythrocyte system lacks the citric acid cycle and metabolises sugars partly to lactic acid through glycolysis and partly to CO_2 through the hexose monophosphate shunt. However, the response of erythrocyte carbohydrate metabolism to insulin has apparently not yet been investigated. The present communication deals with the action of insulin on the formation of lactic acid and CO_2 from glucose in the erythrocytes of normal and diabetic (alloxan-induced) rats in vitro.

Materials and methods

Experimental animals

Male Sprague-Dawley rats (200–250 g) were fed a Hindustan Liver Pellet diet and maintained in the animal house of the Central Drug Research Institute. Diabetes was induced by treatment with alloxan as reported earlier [2]. The mean blood glucose value of diabetic rats was 19.44 ± 1.1 mmol/l of blood.

D-[U-^{14}C]-glucose (specific activity 255 mCi/mmol) was obtained from Bhabha Atomic Research Centre (Bombay, India) and porcine insulin was procured from the Sigma Chemical Company (St. Louis, Mo, USA). All other chemicals were of analytical grade and were provided by BDH or E. Merck (Bombay, India).

Preparation of erythrocyte suspension and glucose oxidation studies

The preparation of the erythrocyte suspension and the measurement of $^{14}CO_2$ production from ^{14}C-(U)-glucose was carried out according to the method of Kelman et al. [6] using Warburg flasks. To 2 ml of erythrocyte suspension in Krebs-Ringer phosphate buffer (without glucose) was added the required insulin concentration. After preincubation with the hormone, the reaction was started by adding 5 mmol/l glucose supplemented with 0.5 μCi ^{14}C-(U)-glucose (specific activity 255 mCi/mmol). The reaction was stopped by the addition of 0.6 ml of 35% perchloric acid. Appropriate controls in which perchloric acid was added before the addition of glucose were always simultaneously set up.

Lactic acid estimation

The perchloric acid-treated cells from the flasks were transferred to centrifuge tubes and made up to 5 ml with distilled water. After centrifugation at 400 *g* for 10 min, 1 ml of the supernatant was taken and lactic acid estimated using the colorimetric method of Barker and Summerson [7].

Statistical analysis

All results are presented as mean ± SD of 4 experiments. The significance of difference was evaluated by Student's t-test.

Results

Action of insulin on CO_2 and lactic acid generation from glucose was studied under two sets of conditions.

Table 1. Effect of insulin on $^{14}CO_2$ production from ^{14}C-(U)-glucose in erythrocytes of normal and diabetic rats

Concentration of insulin (mol/l)	Normal rats		Diabetic rats	
	Without preincubation	With preincubation	Without preincubation	With preincubation
0	(1.0 ±0.15)[a] 1.03±0.095	(0.98±0.08) 1.00±0.08	(0.75±0.12) 0.73±0.13	(0.79±0.09) 0.72±0.10
10^{-10}	1.00±0.10 $p>0.05$	1.18±0.13 $p>0.05$	0.76±0.09 $p>0.05$	0.92±0.13 $p>0.05$
10^{-9}	1.02±0.11 $p>0.05$	1.29±0.18 $p<0.05$	0.72±0.10 $p>0.05$	0.98±0.09 $p<0.01$
10^{-8}	1.07±0.16 $p>0.05$	1.34±0.15 $p<0.01$	0.78±0.11 $p>0.05$	1.03±0.18 $p<0.05$
10^{-7}	1.09±0.14 $p>0.05$ (1.11±0.14)	1.41±0.18 $p<0.01$ (0.94±0.08)	0.81±0.12 $p>0.05$ (0.74±0.07)	1.08±0.19 $p<0.02$ (0.83±0.05)

[a] All data are relative to this value. The values in parentheses represent the data for normal erythrocytes while the other data are for starved cells

Table 2. Effect of insulin on increase in lactic acid levels in erythrocytes of normal and diabetic rats

Concentration of insulin (mol/l)	Normal rats		Diabetic rats	
	Without preincubation	With preincubation	Without preincubation	With preincubation
0	(1.00±0.09)[a] 1.05±0.05	(1.11±0.10) 1.01±0.06	(0.80±0.07) 0.81±0.08	(0.81±0.08) 0.80±0.09
10^{-10}	0.99±0.06 $p>0.05$	1.20±0.10 $p<0.02$	0.76±0.04 $p>0.05$	1.00±0.08 $p<0.02$
10^{-9}	1.07±0.09 $p>0.05$	1.42±0.16 $p<0.005$	0.80±0.08 $p>0.05$	1.16±0.10 $p<0.005$
10^{-8}	1.08±0.09 $p>0.05$	1.55±0.15 $p<0.001$	0.85±0.10 $p>0.05$	1.34±0.14 $p<0.001$
10^{-7}	1.11±0.11 $p>0.05$ (1.01±0.06)	1.62±0.14 $p<0.001$ (1.06±0.05)	0.84±0.09 $p>0.05$ (0.85±0.04)	1.43±0.13 $p<0.001$ (0.78±0.09)

[a] All data are relative to this value. The values in parentheses represent the data for normal erythrocytes while the other data are for starved cells

Either the hormone and ^{14}C-(U)-glucose were added simultaneously (designated 'without preincubation' in Tables), or cells were incubated at 37 °C with the hormone for 1 h prior to addition of glucose, followed by further incubation for 1 h (designated 'with preincubation' in Tables). In each case perchloric acid was added 1 h after addition of glucose, and the production of $^{14}CO_2$ and lactic acid were estimated.

The results suggested that the CO_2 output, which in this system is a measure of the hexose-monophosphate shunt, is impaired by 20–25% in the diabetic animals ($p<0.05$) (Table 1). Insulin, whether supplied 1 h before or along with ^{14}C-glucose did not exercise any significant ($p>0.05$) influence on $^{14}CO_2$ production in either group (Table 1).

The initial lactic acid content of the normal (non-starved) erythrocyte suspension in each flask (see Materials and methods) was found to be 82±8 μg, which increased to 181±14 μg during 1 h incubation with exogenous glucose, presumably due to lactic acid formation through glycolysis. Essentially similar results were obtained with erythrocytes of diabetic rats except that the lactic acid values were lower both before and after metabolism of exogenous glucose (Table 2). Like CO_2 production, the lactic acid increase was not significantly ($p>0.05$) influenced by insulin under the above conditions (Table 2); nor did preincubation with different insulin concentrations bring about any significant ($p>0.05$) change in the basal lactic acid levels (data not presented).

In another series of experiments the erythrocytes were preincubated for 1 h without any exogenous glucose or insulin and the effect of insulin on $^{14}CO_2$ production and lactic acid levels were studied. Both $^{14}CO_2$ production and lactic acid increase were now found to be sensitive to insulin. However, the stimulatory response of insulin manifested itself only after preincubation of the cells with the hormone before the addition of glucose (Tables 1 and 2). These responses depended on the hormone concentration and were higher in the erythrocytes of diabetic animals when expressed as percentage increase over respective non-insulin controls. These increases were statistically significant in both groups ($p<0.01$–0.05) except at 10^{-10} mol/l in the case of CO_2 production ($p>0.05$) (Table 1).

Discussion

The above results demonstrate that although the metabolism of glucose both through the glycolytic and hexose monophosphate shunt mechanisms is unresponsive to insulin in normal erythrocytes, it is rendered hormone sensitive by a brief incubation of these cells in a glucose and insulin-deficient environment. Goodman and Cairo [8] reported that preincubation of rat adipose tissue resulted in marked stimulation of the insulin-like response to growth hormone, and that to insulin itself, of CO_2 production from glucose, although the latter was not found to be as consistent.

The observation that stimulation of CO_2 and lactic acid generation from glucose by starved erythrocytes required their preincubation with insulin suggests that both these effects may be receptor mediated. One possible explanation for insensitivity of normal (unstarved) cells could thus be inadequate availability of vacant hormone receptor sites, due to their prior interaction with insulin in circulation. On incubation in the absence of the hormone, their availability may increase through receptor recyclisation [9] and/or degradation of receptor-bound hormone through the action of insulin degrading enzymes [10]. However, if this postulate

holds true, one would expect erythrocytes to be prepared in insulin-activated state, and hence some reduction in their basal capacity to metabolise glucose on being incubated in insulin deficient medium. This was not generally observed in our experiments and further work is thus needed to satisfactorily explain the present observations.

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Dr. P. Sagar
Biochemistry Division
Central Drug Research Institute
Lucknow
India