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Insulin Effects on Glucose Utilization by Human Lymphocytes

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In recently isolated human lymphocytes, no effect of insulin on (U-^{14}C) glucose incorporation into CO_2, triglycerides and glycogen, was found over a wide range of insulin concentration. The rates of glucose oxidation, synthesis of triglycerides and synthesis of glycogen are much lower than those observed in chicken and rat adipocytes. The authors discard the possibility of using the human lymphocyte as an instrument in the study of the insulin action on normal and altered human physiology.

As it is generally accepted now, the initial stage of the biological action of insulin is its binding to specific protein receptors situated at the surface of the cellular membrane (10, 23, 26).

Up till this point, insulin receptor studies in typically target tissues, as in liver and adipose tissue, have been subjected to extensive examination (6-9, 12, 13, 21). Unmistakable biological effects have been found in these tissues as a result of physiological doses of insulin. Thus, their effects can be considered as instrumental in the understanding of the properties of insulin receptors in certain endocrine disorders o various organisms.

Working with isolated cells from these tissues presents a series of difficulties. The preparation of these cells is based largely on enzymatic digestion methods and on the mechanical disruption of the tissue. These methods present additional problems such as the presence of contaminating enzymes, the alteration of the plasmatic membrane, cellular heterogeneousness, etc., which can alter the number of target cell receptors with the resulting variation in the biological response (19). In the case of studies carried out on humans, one encounters the additional disavantage of not being able to easily obtain samples of tissues.

It is for this reason that an easily accessible tissue has been sought, one which prevents as far as possible the aforementioned limitations and which is instrumental in understanding human illnesses characterized by an altered function of the insulin receptors.

With respect to this, some authors (24)

established that the B human peripheral lymphocyte is found to be equipped with specific insulin receptors.

However, there are dissenting data maintaining that the lymphocyte, recently isolated from human blood, does not contain specific binding sites for insulin (22). Until now, the role played by the lymphocyte has not been sufficiently clarified.

This study was therefore designed, to examine the possible effects of physiological doses of insulin on the glucose metabolism in the human lymphocyte, and to clarify the possibility of using the lymphocyte as an instrument in the study of the effect of insulin on normal and altered human physiology.

Materials and Methods

Bovine insulin, Eagle's MEN medium and PHA were purchased from Wellcome Research Laboratories (Beckenham, Kent, U.K.), (U-^{14}C) glucose (328 mCi/mmol) and (^{14}C) thymidine (59 mCi/mmol) were purchased from the Radiochemical Centre (Amersham, U.K.). Other chemicals were obtained from commercial sources. All radioactive samples were counted in a LKB-1210 Wallac scintillation spectrometer.

The isolation of lymphocytes. 20-30 ml of heparinized blood was obtained from normal human adults who were subjected to a night's fasting. The blood was then deposited in the Erlenmeyer flask which contained glass balls, and was gently shaken so as to defibrinate it. The same volume of saline solution buffered with phosphate (PBS) was added to this venous defibrinated blood. To separate the lymphocytes, the diluted blood was transferred to a Ficoll-Hipaque gradient so as to break up the cells (4). The cells contained lymphocytes in a 95 % proportion and the rest neutrophils and platelets. The red blood cells were deposited in the bottom of the tube. The viability (97 %-98 %) was determined by: 1) dyeing with trypanblue; 2) blastic transformation and incorporation of (^{14}C) thymidine.

Blastic transformation. Previously washed, the lymphocytes were adjusted to 1×10^6 cells/ml in the Eagle's MEN culture medium, supplemented with 10 % bovine fetal serum (inactivated) and 100 mg/ml of cloxacillin plus ampicillin. The cultures were made in a flat bottomed tube (0.5 × 4 cm) containing 1 ml of cellular suspension and 40 ml/tube of phytohemagglutinin (PHA) at concentrations of 1/4 and 1/8. The 1/1 concentration corresponds to the result obtained from dissolving 50 mg of PHA in 5 ml of PBS. Each dose of PHA was studied twice in each culture. The incubation was carried out in an atmosphere rich in CO_2 (92 % air and 8 % CO_2) at 37° C for 72 hours. After that 0.16 μCi of (^{14}C) thymidine was added. Once again the cultures were gassed and incubated for 16 hours after which they were washed and distributed on circles of Whatman paper (Grade 3 MM). The radioactivity of these circles was measured in accordance with standard technique (11).

The isolation of adipocytes. Subcutaneous adipose tissue was taken from White Leghorn chicks and epididymal adipose tissue from Wistar rats. Adipocytes were prepared as described by RODBELL (25).

Metabolic studies. Lymphocytes were incubated for 3 hours in Krebs-Ringer Bicarbonate medium, containing 2 % bovine serum albumin and in Eagle's medium, containing 0.1 % albumin. Isolated fat cells were incubated for 3 hours in KRB-2 % albumin. (^{14}C) carbon dioxide production from (U-^{14}C) glucose was measured by the GOODRIDGE method (8).

In order to measure the (U-^{14}C) glucose incorporation into triglycerides, the cells

were poured after incubation into 5 ml of chloroform: methanol: HCl (200: 100:1) plus 5 ml of 100 mM HCl (16). The triglyceride extract was dissolved in 1 ml of toluene and 10 ml of scintillation mixture was added containing 0.5 % 2,5-diphenyloxazole (PPO) and 0.01 % p-bis 2-(5-phenyloxazole) (POPOP) in toluene.

The synthesis of glycogen from (U-^{14}C) glucose was measured using the method of WALAAS and WALAAS (28). The precipitated glycogen was dissolved in 1 ml of water and 10 ml of scintillation mixture (1000 ml dioxane, 200 ml 2-methoxyethanol, 4 g PPO/l, 0.2 g POPOP/l) was added prior to counting.

The statistical evaluation of results was performed by variance analysis.

Results

The effect of insulin on the (U-^{14}C) glucose metabolism in human lymphocytes. Insulin has no significant effect on the incorporation of the (U-^{14}C) glucose into CO_2 (table I), or on triglycerides and glycogen in incubated human lymphocytes which were left for 180 minutes in a Krebs-Ringer Bicarbonate medium. Similar results are obtained when the lymphocyte is incubated in Eagle's-0.1 % albumin medium. In these two mediums, the oxidation of glucose to CO_2 and the synthesis of triglycerides and glycogen are not increased by the addition of both physiological and pharmacological doses of insulin. The lack of insulin action in the isolated and incubated lymphocyte could be due to 3 possibilities: that the human lymphocyte does not respond to the insulin; that the methods used to detect the biological effects of the insulin are inadequate; that the incubated lymphocyte, under the experimental conditions of this investigation, undergoes morphological or functional changes which render it unsuitable in the light of the proposed objective.

To discard the latter two possibilities, experiments were carried out (tables II and III).

The effects of insulin on the (U-^{14}C) glucose metabolism in chicken and rat adipocytes (table II). Doses of 100 ng/ml of insulin, increased the synthesis of triglycerides by 187 % in the rat adipocytes. The oxidation of the glucose to CO_2 was increased by 142 % while the glycogen synthesis only increased by 137 %.

With regard to the chicken adipocytes

Table I. *Effect of insulin on the incorporation of (U-^{14}C) glucose into $^{14}CO_2$, triglycerides and glycogen in human lymphocytes.*
Values are given as μmol glucose/l $\times 10^6$ cells/180 min $\times 10^3 \pm$ SEM of 4 determinations.

Insulin (ng/ml)	CO_2		Triglycerides		Glycogen	
	KRB*	EAGLE'S**	KRB	EAGLE'S	KRB	EAGLE'S
0	2.20±0.04	3.86±0.09	0.54±0.09	0.59±0.07	0.25±0.09	0.80±0.55
1		3.62±0.17	0.47±0.03	0.72±0.05	0.21±0.08	0.88±0.66
5		3.59±0.23	0.54±0.10	0.54±0.04	0.25±0.14	0.75±0.48
10	2.42±0.05	3.59±0.04	0.60±0.07	0.55±0.05	0.31±0.11	0.74±0.23
100	2.60±0.20	3.57±0.10	0.56±0.04	0.78±0.03	0.33±0.09	0.79±0.35
1000	2.27±0.18	3.56±0.12	0.60±0.08	0.60±0.07		
10000	2.20±0.06					

* In presence of 2 % - albumin and 6 mM glucose.
** In presence of 0.1 % - albumin and 6 mM glucose.

Table II. *Effect of insulin on the incorporation of (U-^{14}C) glucose into $^{14}CO_2$, triglycerides and glycogen in chicken and rat adipocytes.*
Values are given as μmol glucose/g dry wt of cells/180 min × 10^3 ± SEM of 4 determinations. * $p < 0.01$.

	Medium KRB-2 % albumin, 3 mM glucose					
Insulin (ng/ml)	CO_2	Increase (%)	Triglycerides	Increase (%)	Glycogen	Increase (%)
Rat adipocytes						
0	5017.6 ± 195.5		8454.1 ± 99.5		226.3 ± 14.0	
100	12189.9 ± 294.0*	142.96	24299.1 ± 644.0*	187	537.5 ± 25.5*	137.46
Chicken adipocytes						
0	338.0 ± 10.1		41.8 ± 1.3		3.6 ± 0.1	
100	449.5 ± 20.3*	32.97	54.2 ± 1.8*	22.74	3.9 ± 0.1	

Table III. *Effect of PHA on the incorporation of (^{14}C) thymidine in human lymphocytes.*
Values are given as DPM/1 × 10^6 cells ± SEM of 4 determinations.* $p < 0.01$.

		Lymphocytes preincubated for 180 minutes in:	
PHA concentration	Recently isolated lymphocytes	EAGLE'S medium 0.1 % albumin	KRB 2 % albumin
0	148 ± 28	76 ± 7	103 ± 1
1/8	2846 ± 9*	3548 ± 1719*	5812 ± 1268*
1/4	3410 ± 529*	4736 ± 1099*	7918 ± 530*

however, it was observed that insulin had no significant effect on glycogen synthesis. The increase in the synthesis of triglycerides and in the oxidation of glucose to CO_2 was, owing to the insulin, 27.7 % and 32.9 % respectively. This was inferior to that observed in the rat.

In control conditions, the lipogenesis observed in the chicken was 206 times less than that of the rat. The synthesis of glycogen and the oxidation of glucose to CO_2, as observed in the chicken, was respectively 62 and 15 times less than that observed in the rat.

The effect of PHA on the incorporation of (^{14}C) thymidine in lymphocytes, after being incubated for 180 minutes and recently isolated (table III). In recently isolated lymphocytes, the incorporation of (^{14}C) thymidine increases drastically after the blastic transformation which is produced by the PHA. With larger doses of PHA, the incorporation of (^{14}C) thymidine is increased.

When the lymphocytes, prior to their transformation with PHA, are preincubated for 180 minutes under the same experimental conditions as those under which insulin's effect on the lymphocyte is studied, it is noted that the incorporation of (^{14}C) thymidine is increased in cases where there was blastic transformation, similar to the action of the recently isolated lymphocytes.

Discussion

The results presented here demonstrate that no insulin action on glucose metabolism exists in human lymphocytes at physiological concentration of this hormone.

Although KOBAYASHI and MEEK (20), found an increase in the CO_2 and lactic acid production from (^{14}C) glucose, the long range effect has little physiological value, since using large doses of insulin (10 mg/ml), they only obtained increases of 10 % and 20 % respectively.

Neither the CO_2 production nor the

synthesis of triglycerides or glycogen is increased with physiological or pharmacological doses of insulin (table I). The CO_2 production is very small being 4 times larger than the synthesis of triglycerides. A practically negligible quantity of glycogen (10 times smaller than the oxidation to CO_2) is synthesized from the glucose. All of this strongly suggests that the lymphocyte consumes very little glucose, above all compared to the consumption of other cells as those of adipocyte and liver tissue (5, 6, 25).

The same analytical methods are used in experiments with isolated fat cells from rat and chicken adipose tissue as in experiments with the isolated lymphocyte (table II). In the former, a response to the insulin is observed which is in total agreement with other results already established (17).

When the lymphocytes are subjected to blastic transformation with PHA, after being incubated for 3 hours in both Eagle's and Kregs-Ringer Bicarbonate medium, an increase in the (^{14}C) thymidine incorporation is observed, similar to that observed in the case of recently isolated lymphocytes (table III). This suggests that during the incubation, the lymphocytes do not suffer any form of functional transformation that could cause the lack of insulin action.

On the other hand, the method of isolating lymphocytes renders a total populousness of B plus T lymphocytes in a global proportion of 95 %. Thus, the suggestions of certain investigators (24) are vindicated concerning the type of isolated lymphocytes.

All these results strongly suggest that in our experiments the insufficient insulin action in the isolated and incubated lymphocyte is due fundamentally to the fact that these cells by themselves are incapable of responding to this hormone.

However, there are reports (1, 14, 15, 24) which establish that the human lymphocyte is found to be equipped with specific insulin receptors. Nevertheless it has not been possible to confirm this statement since other authors (22) claim that no such specific insulin receptors exist. Recent studies (2, 3, 27) concerning insulin binding in different blood cells, indicate that it is the monocyte which mostly binds the insulin, and not the B or T lymphocyte. These findings are consistent with our results. Therefore, we would suggest not to use this cell for the study of abnormalities in human pathological states related to the action of insulin.

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Resumen

La insulina, tanto a dosis fisiológicas como farmacológicas no incrementa la incorporación de glucosa-U-C^{14} a triglicéridos, CO_2 y glucógeno en linfocitos humanos recién aislados. La proporción de la oxidación de la glucosa, síntesis de triglicéridos y síntesis de glucógeno en el linfocito es mucho más baja que la que se observa en adipocitos aislados de tejido adiposo de rata o de pollo. Los autores descartan la posibilidad de usar el linfocito humano para el estudio de determinados estados patológicos relacionados con la acción de la insulina.

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