A C T A U N I V E R S I T A T I S L O D Z I E N S I S
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IN VITRO EFFECT OF INSULIN ON THE OSMOTIC FRAGILITY OF HUMAN DIABETIC ERYTHROCYTES

In vitro effect of insulin on the osmotic fragility of human diabetic erythrocytes was investigated. It was found out that small concentrations of insulin (10^{-10} M - 10^{-9} M) increase the osmotic fragility rapidly and at insulin concentrations of 10^{-9} M - 10^{-8} M the top values of hemolysis were observed confirming some earlier fluorescence data.

INTRODUCTION

During its action insulin binds to the specific receptors on the cell surface [1]. Since then, insulin receptors in several cells such as adipocytes, hepatocytes, monocytes, granulocytes, lymphocytes, fibroblasts were found and characterized. It has been recently demonstrated that human [2-5], turkey [6] and sheep [7] ercythrocytes have also specific insulin binding sites. These sites have been characterized [4, 8] and it has been found out that their properties are similar to those of the insulin receptors in other cells. The binding of ^{125}I - labeled insulin to ercythrocytes depends on pH, temperature, time and cell concentration. The existence of insulin receptors in the erythrocyte membrane has been already proved but their significance in those cells still needs elucidation.

Erythrocytes and erythrocyte membranes are very useful for studying insulin interaction with the cellular membrane because of their accessibility and ease of isolation, the more so as there are not significant differences in the hormone action on

the erythrocyte membrane or on the membrane of insulin target cells.

A common accepted model of insulin-membrane interaction is the "receptor-transducer" model which was confirmed by many investigations [9, 10]. According to that model insulin interacts with the membrane binding with the specific membrane receptor without entering the cell. The "receptor-transducer" model needs still the determination of the second messenger of the hormone information. In the case of insulin its action is thqught to be independent of cyclic AMP production in the contrary to hormones such as glucagon and adrenaline, which stimulate adenylate cyclase activity, so the nature of the information transducer remains unknown [11]. One hypothesis postulates that alterations of membrane lipid structure and/or changes in membrane fluidity under influence of insulin action may play a role of a hormonal information transducer [12-15].

The erythrocyte membrane fluidity is associated with the osmotic fragility of the erythrocytes. Osmotic fragility is considered to be a measure of erythrocytes viability dependent on its ability to undergo deformation during passing through blood capillaries. The erythrocyte deformability is determined by the surface area to volume ratio and by the elastic membrane properties which enable erythrocytes to undergo strong deformations without rupturing of the cell membrane.

In the case of the erythrocytes from human suffering from diabetes the decreased deformability was found [16, 17]. Because the diabetic erythrocytes are less deformable they may exert an increased pressure on the blood vessel walls leading to microangiopathy.

The alternations of the dynamic properties of the erythrocyte membrane bilayer in diabetes were also found out. The microviscosity of lipid bilayer [18] measured by the help of the fluorescence polarization technique and viscosity related to lateral diffusion of the fluorescent label in that bilayer [19] had much greater value in the case of the diabetic human erythrocyte membranes indicating an increased rigidity of the hydrophobic membrane region.

In this study an experiment was undertaken to investigate an in vitro influence of insulin on the osmotic fragility of diabetic erythrocytes.

MATERIALS AND METHODS

Blood from individuals suffering from insulin-dependent diabetes was obtained from Diabetological Clinic of Medical Academy of Łódź. The patients were of both sexes, (25-55) years old, treated with insulin. The blood was taken after night fast, without morning dose of insulin, on the 3% sodium citrate. All the patients showed far advanced diabetic complications as polyneuropathy and retinopathy. Disease had a long duration of more than 7 years.

Feshly drawn blood was centrifuged at 3000 rpm for 10 minutes. After removing plasma and leukocyte layer, erythrocytes were further washed three times by phosphate buffered saline (PBS: 0.9% NaCl in 10 mM phosphate buffer, pH 7.4).

Washing was held at temperature 277 K. Erythrocytes were then brought to hematocrit of 0.5. Hematocrit was determined using a microcentrifuge Janetzki TH 12, time of centrifugation 300 s.

Insulin (preparation of insulin maxirapid, producer: "Polfa") was added into erythrocyte suspension of hematocrit 0.5 and samples were incubated at temperature 310 K for 15 minutes. The erythrocyte suspension of the hematocrit 0.5 with or without insulin was next added into NaCl solutions of different salt concentrations (0.38-0.50%) in such amounts in order to obtain hematocrit of 0.005. The final insulin concentrations in samples were $4 \cdot 10^{-10}$ M, $2 \cdot 10^{-9}$ M, $2 \cdot 10^{-8}$ M and $2 \cdot 10^{-7}$ M respectively. The samples were incubated at the temperature 310 K for one hour and then centrifuged at 2000 rpm for 10 minutes. The absorbance of the supernatants (hemolysates) was measured at 542 nm. A degree of hemolysis was calculated assuming that an absorbance obtained after introducing the same amount of erythrocytes into distilled water (total hemolysis) is equal 100%.

RESULTS AND DISCUSSION

The obtained results were characterized by a great dispersion of percent hemolysis values for different individuals, for

Tabela

The effect of an increasing insulin concentrations on the percent of erythrocyte hemolysis value for different NaCl concentrations
(the percent of hemolysis in the absence of insulin was assumed equal 1)

Wpływ wzrastających stężeń insuliny na procentową wartość hemolizy erytrocytów przy różnych stężeniach NaCl
(procent hemolizy bez insuliny przyjęto jako 1)

Insulin concentration [M]	NaCl concentration (%)					
	0.42	0.44	0.46	0.48	$\bar{X}$	S.D.
0.0	1.00	1.00	1.00	1.00	1.00	-
$4.04 \cdot 10^{-10}$	1.08	1.03	1.03	1.09	1.06	0.03
$2.02 \cdot 10^{-9}$	1.10	1.10	1.25	1.17	1.16	0.07
$2.02 \cdot 10^{-8}$	1.06	1.07	1.14	1.14	1.10	0.05
$2.02 \cdot 10^{-7}$	0.99	0.98	1.05	1.02	1.02	0.03

$\bar{X}$ - mean of the percent of erythrocyte hemolysis value for all NaCl concentrations.
S.D. - standard deviation.

the same NaCl concentrations. During the results description it was assumed that the percent of hemolysis value in the absence of insulin is equal 1 and a change of that value caused by insulin of different concentrations was calculated. It enabled a statistical description of results concerning the insulin effect on the osmotic fragility of erythrocytes. The obtained results are given in the table 1, and the Fig. 1 gives their graphic interpretation. Fig. 1 represents a relative, mean for all NaCl concentrations, change of an erythrocyte hemolysis percent ($\%h_x$) versus the percent hemolysis value in the absence of insulin ($\%h_o$), taken for equal 1, i.e. $\%h_x/\%h_o$ vs insulin concentration. An insert shows a curve shape for the lowest insulin concentrations.

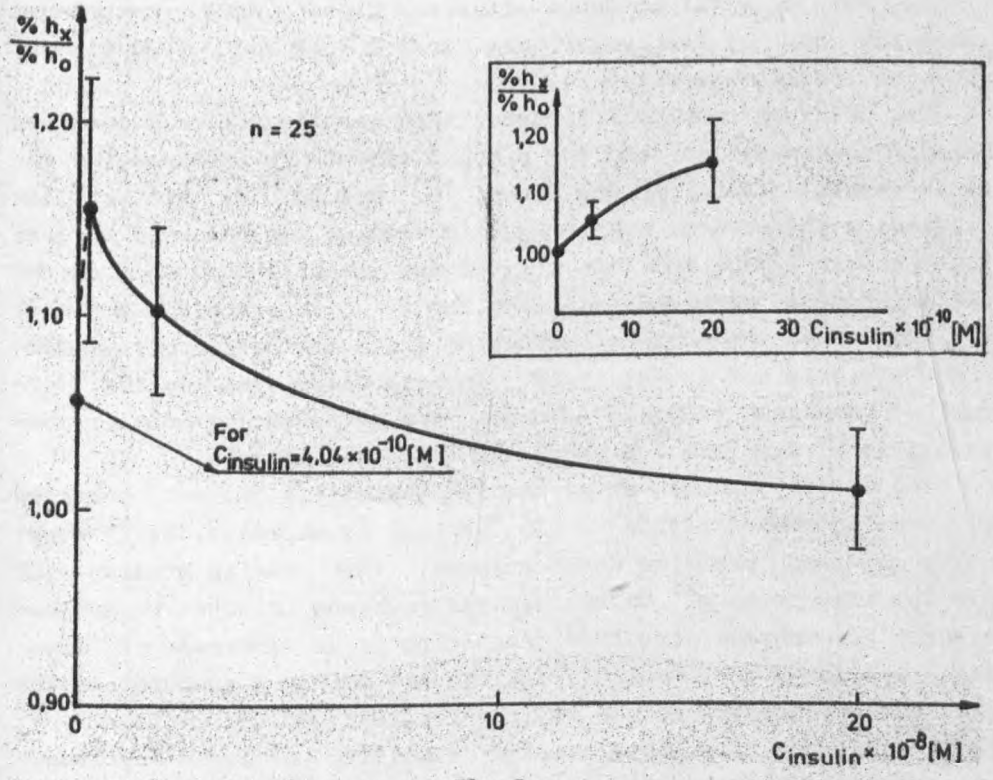

Fig. 1

The effect of an increasing insulin concentrations on the osmotic fragility in diabetic human erythrocytes. The insert shows a curve slope for the lowest insulin concentrations

Wpływ wzrastających stężeń insuliny na wrażliwość osmotyczną cukrzycowych erytrocytów ludzkich. Rysunek wewnętrzny przedstawia nachylenie krzywej dla najniższych stężeń insuliny

The figure indicates, that under the influence of small concentrations of insulin in the range of 10^{-10} M - 10^{-9} M the osmotic fragility increases rapidly reaching a maximum for the insulin concentration equal about 10^{-9} M - 10^{-8} M. For greater insulin concentrations its effect on the osmotic fragility decreases and the percent hemolysis value of erythrocytes trends towards the value observed for the absence of insulin ($\%h_x / \%h_o \rightarrow 1$).

The plots of erythrocyte osmotic fragility curves were also made without insulin and for that insulin concentration when the highest effect of hormone on the investigated value was observed ($2 \cdot 10^{-9}$ M). Curves are given in Fig. 2. The figure shows, that under the insulin action the osmotic fragility curve is parallelly shifted to the right, towards increasing NaCl concentrations. The c_{50} values found from the plots (NaCl concentrations for 50% of hemolysis) are equal 0.436% and 0.443% without and with insulin respectively.

The obtained results indicate, that insulin action depends on hormone concentration and the highest effect is obtained for insulin concentrations in the range 10^{-9} M - 10^{-8} M. The similar saturation phenomenon during insulin action was observed by L u l y et al. [13], who investigated the insulin influence on the rat hepatocyte membrane fluidity and by B r y s z e w s k a et al. [20] when the hormone effect on the human erythrocyte membrane fluidity was investigated. In both cases the greatest effect of membrane viscosity changes was obtained for insulin concentrations equal 10^{-8} M and $5 \cdot 10^{-8}$ M respectively.

The largest change of erythrocyte hemolysis percent observed for insulin concentration $2 \cdot 10^{-9}$ M was equal about 15%. The results obtained prompted us to suppose, that insulin binding with its specific receptor on erythrocyte membrane causes remarkable changes in membrane structure resulting in an increase of osmotic fragility. The osmotic fragility is mainly a function of the initial erythrocyte volume to the critical volume ratio V_i/V_c [21]. Thus the increasing osmotic fragility of human erythrocytes influenced by insulin indicates that the critical erythrocyte volume related to hemolysis diminishes. Influenced by insulin the membrane becomes less resistant to any deformations and bursts more rapidly. The diminished membrane ability for defor-

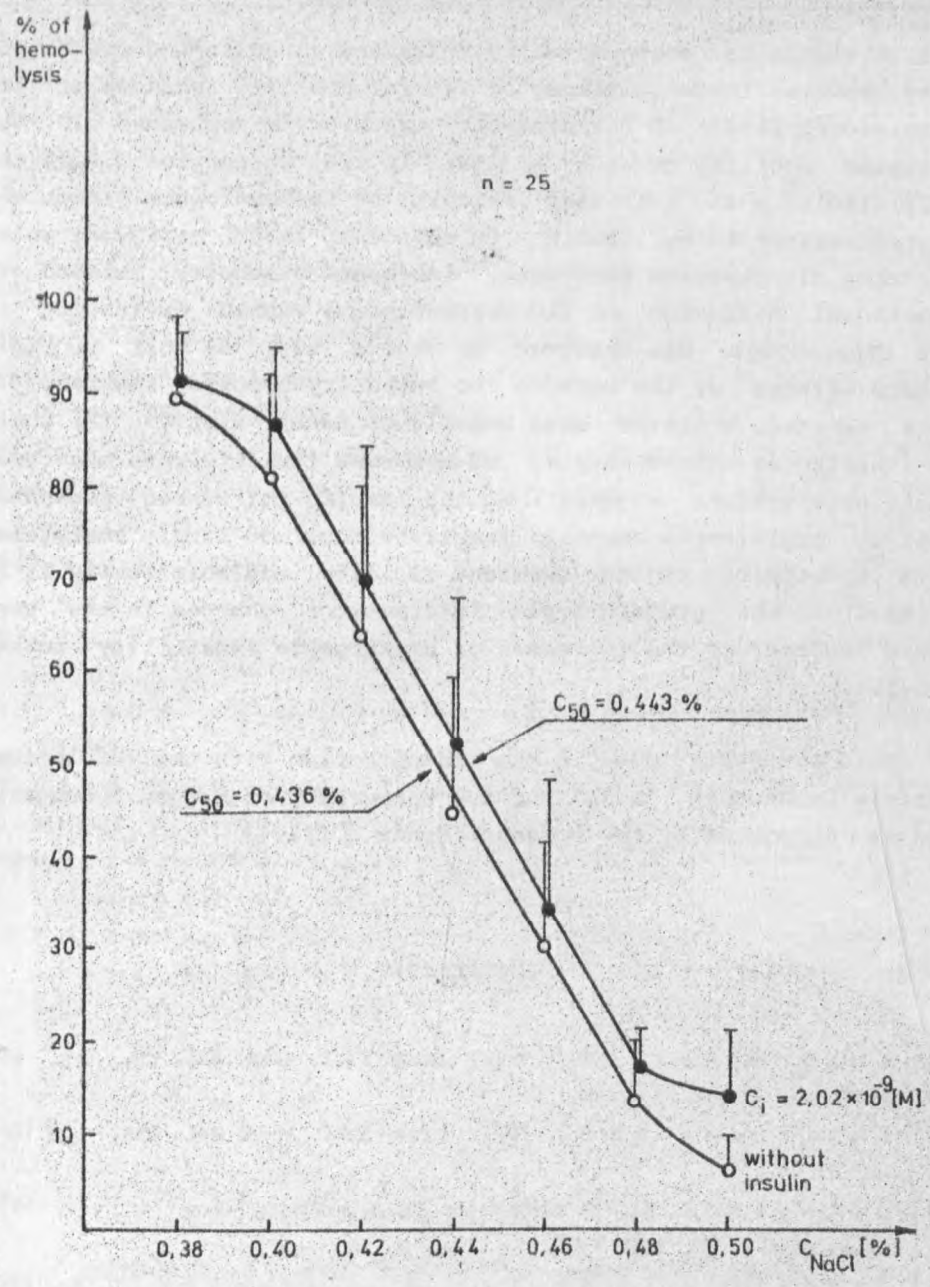

Fig. 2

The osmotic fragility curves of diabetic human erythrocytes under the influence of insulin

Krzywe wrażliwości osmotycznej erytrocytów ludzi chorych na cukrzycę pod wpływem insuliny

mation should be accompanied by increased insulin-induced rigidity. However investigations of lateral mobility changes of the fluorescent label in the diabetic erythrocyte membranes [20] and rotation mobility changes of that in rat adipocytes membrane [12] indicate an increased fluidity of the hydrophobic membrane region influenced by insulin. On the other hand, employing polarization fluorescence technique, increased viscosity related to rotational diffusion of fluorescent probe caused by insulin in rat hepatocytes was observed by others [13], as well as rigiditory effects of the hormone to human erythrocyte membrane [14] were reported. Moreover some unambigues data exist [9, 15] where no insulin-influenced changes of membrane fluidity were observed. These observations suggest that the insulin-influenced augmentation of erythrocyte osmotic fragility does not simply correlates with hydrophobic region membrane fluidity and more likely it is a result of the protein-lipid interactions changes in the membrane leading to the increase of erythrocyte sensibility to hemolysis.

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WPŁYW INSULINY IN VITRO NA WRAŻLIWOŚĆ OSMOTYCZNĄ ERYTROCYTÓW LUDZI CHORYCH NA CUKRZYCĘ

W pracy zbadano wpływ insuliny na wrażliwość osmotyczną erytrocytów ludzi chorych na cukrzycę zależną od insuliny. Stwierdzono, że insulina w ma-

łych stężeniach (10^{-10} M - 10^{-9} M) powoduje duży wzrost wrażliwości osmotycznej erytrocytów. Dla stężeń insuliny równych 10^{-9} M - 10^{-8} M obserwowano największą hemolizę erytrocytów, co potwierdziło uzyskane wcześniej wyniki z pomiarów fluorescencyjnych, dotyczących wpływu tego hormonu na ciekłość błon erytrocytów.