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ULTRASTRUCTURAL CHARACTERISTICS OF INSULIN-RECEPTOR BINDING IN NON-OBESE TYPE 2 DIABETICS WITH NORMAL INSULIN BLOOD LEVEL

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Peripheral insulin resistance (PIR) is a characteristic feature of diabetes mellitus. There is still very little experience with application of electron-microscopic methods for investigation of insulin resistance in type 2 diabetics. The aim of this study is to explore by using an electron- microscopic method the insulin binding, the distribution and internalization ofinsulin-receptor complexes (IRC) in some kinds of blood cells from patients with non-obese type 2 diabetes and normal insulin level. An electron-microscopic method is applied. The marking of IRC is by means of colloidal gold prepared in accordance with G. Frens' technology (1973) modified by M. Horisberger and J. Rosset (1977). Colloidal gold solution of 0,01 % $HAuCl_4$ (Merck) with particle size of 12 nm is used. After a floculation test Insulin Actrapid HM 40 IU/ml, Novo Industri, A/S Denmark with optimal concentration is complexed with colloidal gold. Venous blood cells are incubated with insulin-colloidal gold complexes for 5, 15 and 30 min, respectively, postfixed in 1% osmium tetraoxide and additionally processed for routine electron-microscopic examination with electron microscope JEM 7A. Ten patients with non-obese type 2 diabetes from the Clinic of Endocrinology, Medical University, Varna are studied. The investigation of insulin-receptor binding has been carried out in a state of poor metabolic control on the face of treatment with Glibenclamide in dose up to 20 mg per day. The control group includes 5 healthy subjects. The clinical and biochemical characteristics of the investigated patients are summarized on table 1.

Table 1

Clinical group	n	males	females	age years	BMI kg/m^2	blood sugar mmol/l	IRI mIU/l	HbA_{Ic} %
Type 2 diabetes	10	4	6	58,9	24,8	12,7	13,5	9,3
Controls	5	2	3	56,3	24,5	4,6	18,5	-

IRC are observed on several kinds of blood cells from diabetic patients - erythrocytes, leukocytes, thrombocytes. Control cells from healthy subjects are parallelly studied (fig.1). In all cases the observed IRC labeled with colloidal gold are diminished in number when examined along the cell membrane (fig.2). In most cases gold particles are situated separately and no aggregation is observed.

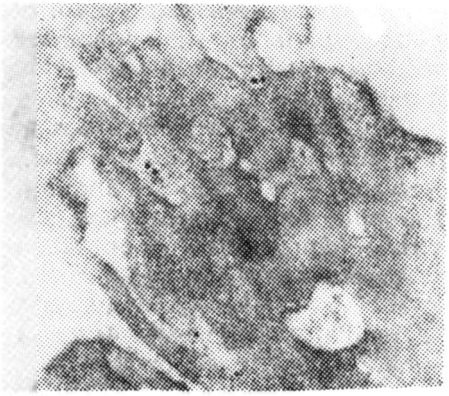

Fig. 1 Magn. x 20 000

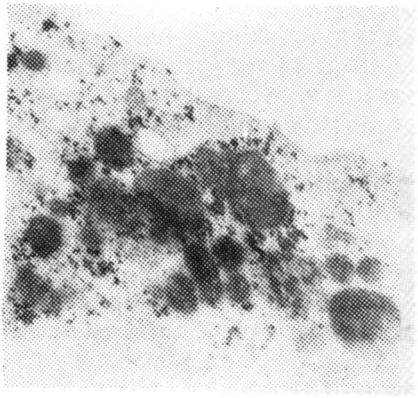

Fig. 2 Magn. x 20 000

In few cases invaginations of the cell membrane enclosing IRC are found. Internalization is decreased and concerns engagement of non-coated vesicles. In spite of the lack of hyperinsulinemia and obesity, clinical and biochemical data in support of PIR are established. Hyperglycemia on the face of normoinsulinemia confirms the exsistance of PIR. Decreased insulin binding and impaired internalization are an eventual expression of receptor and postreceptor defects. Most probably, they both are included in the pathologic events leading to PIR in type 2 non-obese diabetes with normal insulin level.

We conclude that: 1. Colloidal gold is an useful marker for investigation of insulin binding and internalization in blood cells from type 2 non-obese diabetic patients. 2. Insulin-receptor binding and internalization in blood cells from type 2 non-obese diabetics are decreased. 3. In patients with type 2 non-obese diabetes with normal insulin level data supporting PIR are established. 4. PIR in patients with type 2 non-obese diabetes and normal insulin level can most probably be attributed to receptor and postreceptor defects.