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Platelet insulin receptor determination in non-insulin dependent diabetes mellitus

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Summary. The platelet membrane insulin receptors of healthy and non-insulin dependent (type 2) diabetic patients were studied. Receptor number and affinity proved to be decreased in type 2 diabetes mellitus. The changes in platelet insulin receptor characteristics are in good correlation with the alterations reported in other tissues or cells. The possible role of these phenomena in the pathogenesis of disturbed platelet function in diabetics needs further investigation.
Key words. Diabetes mellitus, type 2; platelets, human diabetic; insulin receptor, platelet; platelet insulin receptor.

The demonstration and characterization of human platelet membrane insulin receptors were done by Hajek et al.[1] in 1979. In this paper 125-I-insulin binding of isolated platelets from healthy persons and from non-insulin dependent diabetics (type 2 diabetes mellitus) was compared.

Materials and methods. The healthy control group was composed of 19 persons (male: 10, female: 9); the mean age was 46.4 years. 13 diabetic patients were studied (male: 8, female: 5). They all had the non-insulin dependent form (type 2) of the disease, without severe obesity; none of them received insulin or oral antidiabetic drugs. In the test period the patients had good metabolic control. Their mean age was 53.4 years. The same platelet isolation procedure was performed in the control as in the diabetic group, although the platelet population in diabetes mellitus might be more heterogeneous.

All of the samples were collected during fasting, between 08.00 and 09.00 h. Our method was based upon the one that Hajek[1] had used. Briefly: Blood was collected by vein puncture, and ACD (citric acide, dextrose) solution was used as anticoagulant. Platelet-rich plasma was obtained by centrifugation (15 min, 300 × g, 4 °C). After washing in Tyrode (0, 35% BSA) solution, platelets were resuspended in 'HEPES-buffer' (0.1 M HEPES, 0.12 mM NaCl, 1.2 mM $MgSO_4$, 2.5 mM KCL, 10 mM glucose, 1 mM EDTA, 1% BSA, pH 8.00). For the insulin binding assays a commercially availabe monoiodinated 125-I-insulin was used (MTA 1-RBO-22). 125-I-insulin was added to the suspension of platelets (insulin 100 pg/tube, 1–2 × 10^8 platelets/tube) in 'HEPES' buffer in the presence or absence of unlabeled insulin (0–10 μg/ml). The mixtures were incubated at room temperature for 150 min. After the incubation the platelets were separated by centrifugation, and radioactivity of the platelet sediments was determined.

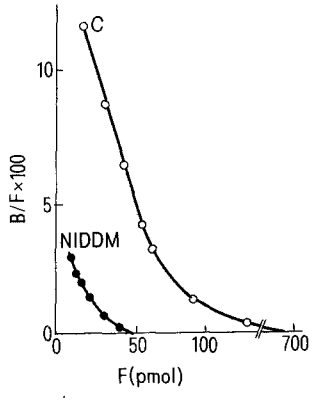
Platelet insulin receptor study

	Receptor/ cell ± SD	$1/CD_{50} \pm 10^7$ mmol/l	Half-maximal displacement nmol/l ± SD
Control	420 ± 116 (110 ± 23)	2.72 ± 0.75	5.98 ± 2.48
Diabetes mellitus type 2 (13 patients)	30 ± 11	1.20 ± 0.42	3.11 ± 1.73

The insulin binding in the presence of 10 μg/ml unlabeled insulin was regarded as nonspecific (2, 1–3, 0%). The results were evaluated by the graphical method of Scatchard[2], using the same conventions (i.e. the negative cooperativity model) as Hajek et al.[1] had done. According to their method only one association constant was given derived from the dissociation constant (CD_{50}) of the whole curve. For a better comparison the half-maximal displacement values were also determined.

Results. The Scatchard-plots derived from our data are in the figure.

The curvilinear shape of the plots appears to be consistent with either the presence of two classes of platelet insulin receptors with different numbers and affinity, or a single population according to the negative cooperativity model. The results shown here, of course, are not suitable for making a decision owing to the difficulties of explanation and interpretation of Scatchard-plots. (In order to make an adequate comparison of our results with those of Hajek et al.[1] we had to use the same mathematical conventions as they had done.) However, the remarkable decrease in the number of affinity of platelet insulin binding sites in the diabetic patients could be clearly demonstrated. In the table the results are shown in numerical form.



Platelet insulin receptor, Scatchard plots. 'F' is the quantity of free, 'B' the quantitiy of bound insulin. C: control group. NIDDM: non-insulin dependent diabetes mellitus.

The number and affinity of platelet insulin receptors in non-insulin dependent diabetes mellitus were significantly reduced. The receptor number in parentheses shows the quantity of binding sites of the low-number–high-affinity receptor class; less than one-third of the control value could be detected. The reduction of the affinity constant and half maximal displacement values demonstrate the decreased affinity of platelet insulin receptors in diabetes mellitus (type 2).

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Emblica officinalis reduces serum, aortic and hepatic cholesterol in rabbits

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Summary. Emblica officinalis reduced serum cholesterol ($p < 0.001$), aortic cholesterol ($p < 0.001$) and hepatic cholesterol ($p < 0.001$) significantly in rabbits. *Emblica officinalis* did not influence euglobulin clot lysis time, platelet adhesiveness or serum triglyceride levels.
Key words. Emblica officinalis; cholesterol; atherosclerosis, rabbits.

Emblica officinalis (family Euphorbiaceae) grows in homeyards and deciduous forests[1]. The fruit (pulp and seeds), bark, roots, flowers and leaves of this plant have been used as ayurvedic medicines. Its pulp contains 13 separable tannins and colloidal complexes and is a rich source of vitamin C[2,3]. In ayurvedic literature it is mentioned that amla *(E. officinalis)* forms one of the important contituents of a drug (Chavanpras) which prevents ageing. This encouraged us to test the anticholesterolaemic effect of the dried pulp of the amla. It was shown in this laboratory that *E. officinalis* reduced serum cholesterol in rabbits[4]. We further showed that the hypocholesterolemic effect of *E. officinalis* was not entirely due to its vitamin C content and we suggested that there might be other substance(s) responsible for that action. We have extended this study further to examine the effect of *E. officinalis* on cholesterol contents of liver and aorta in experimentally produced atherosclerosis in Indian albino rabbits.

Materials and methods. 70 Indian albino rabbits (half male, half female), weighing 1.1–1.6 kg, and aged 8–10 months, were fed daily on 25 g of Bengal gram, 15 g of maize flour, green grass and drinking water ad libitum. Pre-experimental fasting blood samples for estimation of serum lipids, euglobulin clot lysis time and platelet adhesiveness were collected from an ear vein. Each rabbit was fed with cholesterol 0.1 g/kg b.wt in addition to the routine diet to start with for two weeks. On the basis of preliminary cholesterolemic response, b.wt, age and sex, 50 out of the 70 rabbits were randomly allocated into two groups, A and B. Any rabbit showing a very high or low cholesterolemic response was excluded. The animals were treated as follows:

In addition to the diet, group A received cholesterol 0.3 g/kg b.wt and group B received cholesterol in the above dosage plus *E. officinalis* 1 g/kg b.wt (table 1). The pulp separated from the fresh fruits was dried, powdered and then used.

Serum cholesterol[6] and triglyceride[7] were estimated fortnightly. The experiment lasted for only 16 weeks. At the end of the experiment the blood was taken out for estimation of cholesterol, triglyceride, euglobulin clot lysis time[8] and platelet adhesiveness[9]. The rabbits were killed with an overdose of pentobarbitone, and the aortas were examined. The aorta was opened longitudinally from the aortic valve to the iliac arteries and was stained with a mixture of sudan IV and sudan III. After 10–12 min the aorta was washed with tapwater. Atherosclerotic lesions appeared as sudanophilic areas. Sudanophilic areas were traced on to paper and then the tracings from the whole surface were transferred to graph paper and the percentage involvement was calculated. The aortas of five rabbits from each group were kept for histopathological examination and the aortas of 20 rabbits from each group were kept for cholesterol estimation. The lipids were extracted by the method of Folch et al[11]. Paraffin sections were stained with H & E and van Gieson. Frozen sections were cut on a cryostat and stained with a mixture of sudan III and IV. Atherosclerotic involvement was graded according to the WHO recommendations[10]. Livers were removed and weighed, and an aliquot of liver was homogenized in chloroform-methanol (2:1)[11] and the extract used for determination of total cholesterol. The aortas were similarly homogenized in chloroform-methanol for extraction[11]

Table 1. Dietary regime, initial and final b.wts of animals

Groups	Number	Diet	Initial b.wt (g ± SD)	Final b.wt (g ± SD)	Mean weight gain (g)
A	25	Cholesterol 0.3 g/kg b.wt	1553 ± 190	1580 ± 180	27
B	25	Cholesterol in above dosage + *E. officinalis* (Amla) 1 g/kg b.wt	1540 ± 130	1579 ± 120	38.9

Table 2. Mean serum cholesterol and triglyceride levels during the experiment and their significance (mg ± SD)

Weeks on diet	Group A Cholesterol	Triglyceride	Group B Cholesterol	Triglyceride
Initial	74.4 ± 5.7	68.5 ± 2.2	82.4 ± 5.6	72.4 ± 6.4
12th week	593 ± 98.8	81.4 ± 10.8	241.6 ± 20.8	81.4 ± 7.8
16th week	632.8 ± 158	88.2 ± 15.4	116.8 ± 18.6	72.8 ± 14.8
Cholesterol 16th week: A:B $p < 0.001$				