PLATELET GUANYLATE CYCLASE IN HUMAN DIABETICS

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Guanylate cyclase (GC) catalyzes the biosynthesis of cyclic guanosine monophosphate (c-GMP), a powerful regulator of cell metabolism. In human platelets 95% of the GC content is in the soluble form, and possesses high activity [4]. The great interest in GC of human platelets is due to the important role of cGMP in the regulation of platelet aggregation and disaggregation [7, 9, 12]. In diabetes mellitus in most cases hyperaggregation of platelets is is observed, and it is one of the main causes of the angiopathies and of the development of the DBC syndrome [1, 8, 11]. The mechanism of regulation of platelet aggregation and disaggregation and the role of cGMP and GC in this process have not been adequately studied.

Accordingly, in the investigation described below, soluble GC isolated from platelets of healthy individuals and diabetics of types I and II was investigated. The aim of the investigation was to discover changes in the activity and regulation of GC and to detect differences between types I and II of diabetes mellitus as indicators of the heterogeneity of this disease.

EXPERIMENTAL METHOD

Eighteen patients of both sexes with normal body weight, aged from 20 to 45 years, and with types I and II of diabetes of different duration, in the compensation stage were investigated. The blood glucose was measured on a "Beckman" glucose analyzer. Immunoreactive insulin was determined with the aid of standard kits (USSR) or kits for radioimmunoassay of insulin (Hungary).

The control group consisted of 18 healthy subjets of both sexes aged from 20 to 45 years.

Blood from all subjects was taken before breakfast from the cubital vein in a volume of 10 ml; 0.1 M EDTA in a ratio with blood of 1:10 by volume was used as the anticoagulant. Platelets were isolated by the method described previously [3] using "Ficoll-Paque" (Pharmacia, Sweden). A suspension of washed platelets in 50 mM Tris-HCl, pH 7.6, containing 0.2 mM dithiothreitol, was sonicated at 0°C on an MSE-5-78 ultrasonic disintegrator (Great Britain) for 20 sec, then centrifuged for 1 h at 105,000g on a "Beckman L5-65" ultracentrifuge (Austria). The supernatent was used as the GC preparation.

GC activity was determined by the method described previously [3], in the presence of 1 mM GTP and 4 mM $MgCl_2$ or $MnCl_2$. The quantity of cGMP formed during the enzyme reaction (10 min at 37°C) was determined by radioimmunoassay using "cGMP RIA Kits" (Amersham International, Great Britain). In the experiments to determine the activating action of sodium nitroprusside, the GC preparation was preincubated wth 0.1 mM nitroprusside for 50 min at 0°C [3].

EXPERIMENTAL RESULTS

A complex of GTP with the bivalent cation Mg^{++} or Mn^{++} was used as the substrate for GC. GC activity, determined in the presence of Mg^{++}, is basal. Basal activity reflects the degree of oxidation of the enzyme. Reversible oxidation of GC is known to activate the enzyme, whereas

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TABLE 1. Platelet GC Activity in Normal Subjects and Patients with Diabetes of Types I and II (M ± m)

Experimental conditions	Number of patients	GC activity, pmoles cGMP/g protein/min			Ratio of Mn-dependent to Mg-dependent activity	Degree of activation by nitroprusside
		Mn^{2+}	Mg^{2+}			
			without nitroprusside	in presence of 0.1 mM nitroprusside		
Normal	18	409±34	187±12	2115±174	2,16±0,12	11,56±1,07
Diabetes type I	10	326±29	128±9*	859±62*	2,55±0,15	6,70±0,38*
Diabetes type II	8	272±20*	92±4**	336±29**	2,98±0,22*	3,61±0,30**

Legend. *$p < 0.05$ compared with normal, **$p < 0.05$ compared with diabetes type I.

reduction inhibits it [4]. In the presence of Mn^{++}, GC activity always exceeded the basal level because of the oxidizing action of manganese, as a metal with variable valency [4, 5]. Stimulation of GC by manganese under these circumstances depends on the degree of reduction of the enzyme tested.

We compared the properties of GC from platelets from normal blood donors and patients with diabetes of types I and II. Activity of the enzyme was determined in the presence both of 4 mM Mg^{++} and of 4 mM Mn^{++}; this concentration of cations is optimal [3]. Reduction of the basal activity of GC was found in platelets of diabetics (Table 1) by 30 and 50% in types I and II respectively. Activity of Mn-dependent GC also was depressed, but by a lesser degree than the basal level. The ratio between Mn-dependent and Mg-dependent enzyme activity is a coefficient which reflects the ability of the enzyme to be activated by manganese [3]; it is raised in platelets from diabetics, more especially those with type II. The results suggest that the fall in GC activity of the platelets in diabetes may be connected with transition of the enzyme into a more reduced state.

An important criterion for assessment of the functional state of GC is the ability of the enzyme to be activated by NO-containing compounds and, in particular, by sodium nitroprusside [4, 6, 9]. Stimulation of GC by NO-compounds is due to transfer of the free-radical NO group to heme, which is the prosthetic group of GC. Under these circumstances a nitrosylheme is formed, closely similar in structure to protoporphyrin IX, one of the most powerful activators of GC [6].

We found a marked decrease in the degree of activation of GC by nitroprusside in platelets from diabetics (Table 1). Both the absolute value of the stimulated GC activity and the coefficient of activation (the ratio between values of Mg-dependent activity of the enzyme in the presence and absence of 0.1 mM sodium nitroprusside) were reduced. In type II diabetes, the fall of these parameters took place more suddenly than in type I.

One of the causes of the observed reduction of the degree of activation of GC by sodium nitroprusside in diabetes could be intensification of free-radical reactions as a result of intensification of lipid peroxidation, which is observed in this disease [2]. In that case the lesser degree of activation of GC by nitroprusside in diabetes of type I compared with type II may have been due to insulin therapy [10].

On the other hand, since the action of sodium nitroprusside on GC is realized through the formation of an NO-heme complex, it can be tentatively suggested that in diabetes the heme is detached from the protein part of the GC molecule (for example, through labilization of the bond [6]) and the heme-deficiency thereby arising is responsible for reduction of the degree of activation of GC by nitroprusside. With this interpretation it must be assumed that heme-deficiency of GC is more marked in type II than type I diabetes.

The results are thus evidence of a decrease both in the activity and in the degree of activation of GC of human platelets in diabetes, and the abnormalities are greater in type II than in type I diabetes. Changes which we found in the properties of GC, especially the change in reactivity of the enzyme, may cause distortion of those physiological functions of the platelets which are under cGMP control and, in particular, regulation of the process of platelet aggregation and disaggregation [7, 9, 12].

LITERATURE CITED

1. A. S. Efimov, Ya. L. Germanyuk, and S. G. Genes, Diabetes Mellitus [in Russian], Kiev (1983).

2. L. K. Starosel'tseva, E. S. Kosilova, T. F. Smirnova, et al., Probl. Endokrinol., 32, No. 1, 19 (1986).
3. Yu. Yu. Chirkov, I. A. Tyshchik, N. N. Belushkina, and I. S. Severina, Biokhimiya, 52, No. 6, 956 (1987).
4. E. Bohme, R. Gerzer, C. Grossman, et al., Hormones and Cell Regulation, Vol. 7, Amsterdam (1983), pp. 147-161.
5. J. M. Braughler, Biochim. Biophys. Acta, 616, 94 (1980).
6. L. J. Ignarro, J. B. Adams, P. M. Horwitz, and K. S. Wood, J. Biol. Chem., 261, 4997 (1986).
7. B. Karrenbrock, R. Grewe, K. Loeschke, and R. Gerzer, Pathol. Biol. 35, 251 (1987).
8. H. C. Kwann, J. A. Colwell, S. Cruz, et al., J. Lab. Clin. Med., 80, 236 (1972).
9. B. T. Mellion, L. J. Ignarro, E. H. Ohlstein, et al., Blood, 57, 946 (1981).
10. S. P. Muckerjee, Biochem. Pharmacol., 27, 2589 (1978).
11. A. J. Schafer and R. I. Handin, Prog. Cardiovasc. Dis., 22, 31 (1979).
12. M. L. Steer and E. W. Salzman, Adv. Cycl. Nucleot. Res., 12, 71 (1980).

RED LIGHT OF A HELIUM-NEON LASER REACTIVATES SUPEROXIDE DISMUTASE

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Irradiation with light from a helium-neon laser (HNL) is being used on an increasingly wide scale in the treatment of diseases connected with ischemia, hypoxia, and various inflammatory processes [3]. The mechanism of this broad action of HNL is not yet understood. It is evident that the radiation can act only on those components of the living cell which absorb light. The wavelength of HNL radiation is 632.8 nm. The most important oxidoreductases absorb in this region: catalase, superoxide dismutase (SOD), cytochrome C oxidase, ceruloplasmin, etc. [4]. Some workers have recorded activation of certain metalloenzymes by HNL light [1, 2, 6], but no action of laser radiation has hitherto been found on native SOD and ceruloplasmin [9, 13].

To understand the mechanism of action of laser light on biological objects, one fact appears to be important, namely that HNL radiation does not affect normally functioning cells, but has a marked action in cases associated with a pathological process. Damage to the body cells under these circumstances may arise under the influence of active forms of oxygen [14]. In these cases, administration of protective enzymes, notably SOD, has a marked therapeutic effect [15]. This effect may perhaps be connected with the fact that the intrinsic SOD of the cells may be partially inactivated in a pathological focus, where the pH is lowered [5]. In experiments with isolated SOD, disturbance of the structure of the enzyme was observed in an acid medium as shown by changes in optical and paramagnetic properties [12]. The question arises whether the therapeutic action of laser radiation may be connected with photoreactivation of enzymes reversibly inhibited in the pathological focus and, in particular, SOD.

The investigation described below showed that reversible inhibition of the enzyme takes place in an acid medium, and that HNL radiation can reactivate SOD. To shed light on the possible mechanism of this process, it was decided to study the activity and absorption and EPR spectra of SOD and of complexes of copper with histidine, which simulate the structure of the active center of the enzyme.

EXPERIMENTAL METHOD

SOD from bovine erythrocytes (from "Boehringer") was dissolved in 10 mM Tris-HCl buffer, pH 5.9, 7.4, and 8.2. Complexes of copper and histidine were prepared by dissolving 1 mM $CuCl_2$

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