Decreased Numbers of Platelet α-Adrenergic Binding Sites in Diabetes Mellitus

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Eleven men with diabetes mellitus were compared with 45 male controls for platelet α-adrenergic binding sites by using [^{3}H]dihydroergocryptine (DHE) as the radioligand antagonist. There was no difference between the two for binding affinity, but the number of sites was 430 ± 30 ($\bar{x}$ ± SEM) for diabetic subjects and 574 ± 29 for controls (P = .005). Decreased sites were related to increased glycosylated hemoglobin levels (P = .002). There was no relationship between the decreased sites and catecholamine levels, duration of disease, body weight, or fasting blood sugar. Hence, binding sites were inversely related to control, but further studies are needed to define the pathophysiologic significance of this.
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People with diabetes mellitus have increased morbidity and mortality from vascular disease. The reason for this has remained obscure, but platelets have been implicated as contributors to this pathologic process.[1] A tendency for platelets to hyperaggregate has been demonstrated in diabetes, and this has been related to control.[2] However, no one has examined platelet receptors as initiators of the aggregation response to see if there are any abnormalities that may be related to control.[3] Hence, we evaluated a group of subjects with diabetes for number and affinity of platelet α-adrenergic binding sites by using [^{3}H]-dihydroergocryptine (DHE) as the radioligand antagonist.

METHODS

Eleven men with diabetes mellitus were studied. Three had clinical cardiovascular autonomic neuropathy, but none had vascular complications or evidence of any other systemic disease. The known duration of diabetes was 100 ± 30 mo ($\bar{x}$ ± SEM). Six had not received any drug treatment, but five had been on insulin. These subjects were compared with a control group, which consisted of 45 men without evidence of systemic disease. These controls and the three diabetic subjects with autonomic neuropathy have been reported elsewhere.[4] No one had taken any medication except insulin for at least 1 wk before the study.

The age, percent body weight, fasting blood glucose, glycosylated hemoglobin, catecholamines, and number and affinity of α-adrenergic binding sites per platelet were determined for all subjects. Glucose was measured by the oxidative method, glycosylated hemoglobin by a colorimetric technique (normal <10%)[5] and epinephrine and norepinephrine by the single-isotope enzymatic derivative method.[6] DHE was used as the radioligand antagonist to determine platelet binding number and receptor affinity as previously described.[4]

All studies were performed in the special studies unit at the VA Medical Center, Seattle, Washington, after approval by the Human Studies Committee and obtainment of informed consent. For catecholamine and receptor studies, each subject fasted and refrained from use of cigarettes and insulin from the midnight preceding the study until after completion. All studies were done at approximately 8:00 a.m. Subjects were recumbent, and a 19-gauge butterfly needle was inserted in the antecubital vein. Patency was maintained by slow infusion of 0.9% sodium chloride. After a half-hour rest, two 2.5-ml samples drawn 5 min apart were analyzed for basal norepinephrine and epinephrine levels. The final value for each catecholamine was the average of these two samples. Then 250 ml blood was drawn for platelet receptor analysis. Blood was also drawn for fasting blood sugar and glycosylated hemoglobin. Population means were compared by nonpaired two-tailed t test. Relationships between binding sites and other parameters were analyzed by linear regression.

RESULTS

Data are summarized in Table 1. The total number of binding sites per platelet was decreased in subjects with diabetes mel-

TABLE 1
Comparison of controls and diabetic subjects ($\bar{x}$ ± SEM)

	Age (yr)	% Ideal body weight	Norepinephrine (pg/ml)	Epinephrine (pg/ml)	Sites per platelet	Affinity (nM)	Fasting plasma glucose (mg/dl)	Glycosylated hemoglobin (%)
Normals (N = 45)	38 ± 2	113 ± 2	277 ± 20	49 ± 6	574 ± 29	5.44 ± .32	90 ± 1	8.0 ± 0.1
Range	19–72	91–147	70–640	0–160	225–1096	7.04–10.10	63–111	6.0–9.0
Diabetic subjects (N = 11)	53 ± 4	121 ± 8	212 ± 28	55 ± 8	430 ± 40	4.8 ± 0.53	218 ± 28	13.7 ± 0.9
Range	35–64	77–165	110–440	30–100	242–647	1.90–7.74	71–376	11.4–20.0
Value	<.001	NS	NS	NS	.005	NS	<.001	<.001

NS, not statistically significant.

litus compared with controls (P = .005; Figure 1), but there was no difference in binding affinity. There was a significant difference between the two for age; however, there was no relationship between age and binding sites for either group. Moreover, there was good linear correlation (P = .002) in diabetic subjects between binding sites and glycosylated hemoglobin (y = 29.3, x ± 816.9; r = 0.64, P = .002). No relationship between sites and norepinephrine, epinephrine, duration of disease, fasting blood sugar, or percent ideal body weight was found.

DISCUSSION

We found the number of binding sites per platelet to be decreased in subjects with diabetes mellitus, and this was related to glycosylated hemoglobin. How these sites were affected by glucose metabolism was unclear. It has been suggested that lymphocyte receptors may undergo structural changes through glycosylation in diabetes.[7] It is possible that the platelet α-adrenergic receptors are glycosylated, causing decreased binding by radioligand. Glycosylation has also been shown to cause cross-linking of proteins and impairment of nucleic acid function,[8] which could result in altered receptors or a decrease in their production.

The fact that we found no relationship between sites and fasting blood sugar may have been a result of our protocol for doing catecholamine levels. This required that insulin be discontinued by midnight before a morning study. Therefore, there may have been atypical glucose changes in our subjects. Because glycosylated hemoglobin reflects glucose control over several months,[9] the time course for sites to change in relation to glucose requires further study.

We used [^{3}H]DHE as the radioligand antagonist. This has been criticized as being a nonspecific α-adrenergic radioligand lacking in reproducibility, compared with other more specific α-ligands such as [^{3}H]yohimbine.[10,11] However, we have found excellent correlation between DHE and [^{3}H]yohimbine for platelets in our laboratory.[4]

Finally, if platelets in diabetes mellitus are hypersensitive, as suggested,[12] one may have suspected that the binding sites or affinity would have been increased. Although we found the number of sites to be decreased, the functional significance of this remains to be investigated.

Hence, we found a decreased number of binding sites per platelet in subjects with diabetes mellitus, and this was inversely related to their glycosylated hemoglobin level. The decreased sites appear, then, to be a consequence of poor control. Further study is needed to determine the pathophysiologic significance of this.

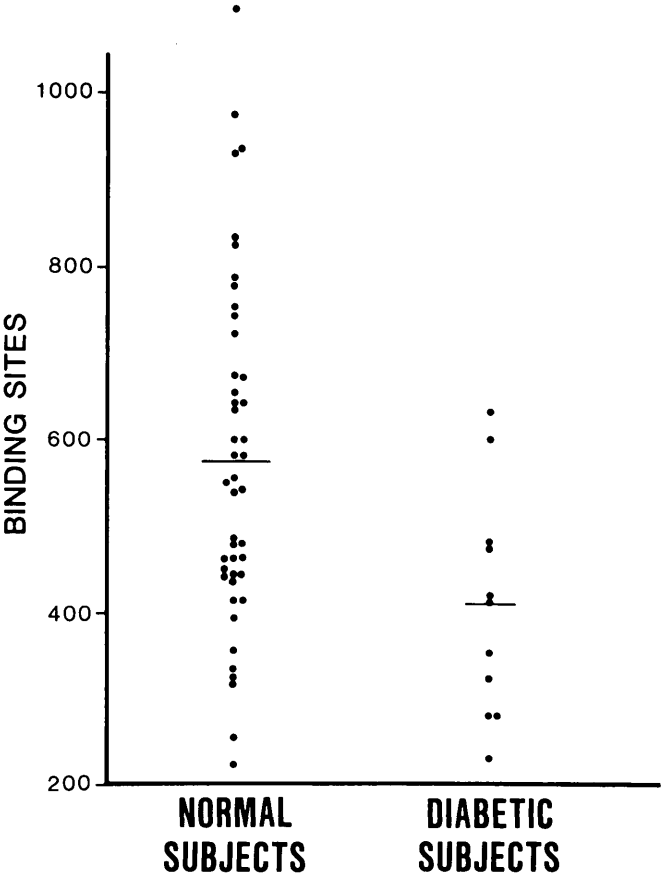

FIG. 1. Number of binding sites per platelet for normal (574 ± 29 SEM) and diabetic (430 ± 40 SEM) subjects is shown. The 2 groups were statistically different at P = .005.

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