Low Levels of Intraplatelet cGMP in IDDM

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OBJECTIVE — To determine the levels of intraplatelet cGMP, an index of activity of the antiaggregatory nitric oxide pathway, in IDDM patients.

RESEARCH DESIGN AND METHODS — We measured intraplatelet and plasmatic cGMP levels in 22 IDDM patients and 22 age- and sex-matched control subjects.

RESULTS — Intraplatelet cGMP levels decreased in the IDDM patients (0.32 ± 0.16 pmol/10^9 platelets) when compared with the control group (0.52 ± 0.32 pmol/10^9 platelets), $P = 0.032$. Plasmatic cGMP levels were not significantly different between groups. Intraplatelet cGMP levels correlated negatively with the duration of the disease ($r = -0.43$, $P < 0.05$).

CONCLUSIONS — IDDM patients have lower levels of intraplatelet cGMP, which may be responsible in part for their platelet hyperactivity.

Increased platelet reactivity to different aggregating agents has been demonstrated in diabetic patients, and this disturbance may be involved in the pathogenesis of macro- and microangiopathy (1). Different mechanisms have been proposed to explain platelet hyperactivity in diabetes (1). Nitric oxide, produced by the vascular endothelial cells, reaches the platelets as they pass through the vascular beds, increasing their cGMP levels and inhibiting platelet activation (2–4). In experimental (5) and human (6) diabetes, studies have found the capacity of the vessel wall to synthesize nitric oxide is reduced.

To test the hypothesis that the antiaggregatory nitric oxide-cGMP pathway is reduced in diabetes, we measured intraplatelet cGMP levels in a group of IDDM patients. We also measured plasmatic cGMP levels, which express ANP function (7), and extracellular calcium and magnesium levels, which are crucial for a physiological production of endothelial nitric oxide (2).

RESEARCH DESIGN AND METHODS

— We studied 22 IDDM outpatients (12 men, 10 women). All gave informed consent. None had other concomitant disease nor was receiving medication other than insulin. None was alcohol-dependent nor had ingested alcohol during the 24 h before the study. None had any infectious episode in the previous 2 wk nor had a serious metabolic decompensation in the previous 3 mo. Patients with serum creatinine levels >105 μM, dBP >90 mmHg, or macrovascular complications were excluded. Six patients had retinopathy (3 proliferative, 3 simple). Three patients had microalbuminuria. None had autonomic neuropathy. The control group comprised 22 age- and sex-matched nonhypertensive, healthy volunteer subjects.

We collected fasting blood samples into 0.105 M sodium citrate (4:1) from the cubital vein before the morning injection of insulin. Neither smoking nor exercise was allowed before taking the samples. PRP was obtained with centrifugation (560 *g*, 10 min, 4°C), and 100 μM 3-IBMX was added to inhibit the activity of cyclic nucleotide PDE. We counted the number of platelets with a Coulter S+ Counter (Coulter, Hialeah, FL) and centrifuged (2500 *g*, 3 min, 4°C) 1-ml aliquots of PRP to obtain the platelet pellet. The supernatant PPP was used to measure plasmatic cGMP. We added 1 ml 6% $HClO_4$ to the pellet, then vortexed and centrifuged it at 2500 *g* for 15 min at 4°C. The aqueous phase was assayed for cGMP with RIA (Advanced Magnetics, Cambridge, MA) (4). Circulating plasmatic cGMP was measured in PPP with the same RIA after protein precipitation with 100 μl 20% $HClO_4$ and neutralization with 200 μl 1.08 MK_3PO_4. RIA sensitivity was 3 fM.

The same assay determined both

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IDDM, INSULIN-DEPENDENT DIABETES MELLITUS; ANP, ATRIAL NATRIURETIC PEPTIDE; dBP, DIASTOLIC BLOOD PRESSURE; PRP, PLATELET-RICH PLASMA; IBMX, ISOBUTYLMETHYLXANTINE; PPP, PLATELET POOR PLASMA; RIA, RADIOIMMUNOASSAY; CV, COEFFICIENT OF VARIATION; HPLC, HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY; PTH, PARATHYROID HORMONE; PDE, PHOSPHODIESTERASE; STZ, STREPTOZOCIN; BMI, BODY MASS INDEX.

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Table 1—*Clinical and biochemical characteristics of diabetic and control groups*

	CONTROL GROUP	DIABETIC GROUP	*P* VALUE*
n	22	22	
SEX (M/F)	12/10	12/10	
AGE (YR)	32.6 ± 6.8(23–45)	33 ± 8.9(20–47)	NS
BMI (KG/M^2)	24.3 ± 1(20–25.3)	22.5 ± 1.5(19.2–24)	NS
DURATION OF DIABETES (YR)	—	11.4 ± 7.4(0.6–29)	—
INSULIN DOSE (U/DAY)	—	42 ± 15(20–72)	—
GLYCEMIA (MM)	4.7 ± 0.6(3.8–5.9)	8.7 ± 3.9(3.5–21)	0.0001
HbA_{1c} (%)	4.3 ± 0.5(3.7–5.8)	7 ± 1.1(4.9–9.6)	0.0001
CHOLESTEROL (MM)	4.9 ± 0.96(3.3–6.4)	4.7 ± 0.8(3.5–6.2)	NS
MAGNESIUM (MM)	0.79 ± 0.1(0.61–1)	0.72 ± 0.06(0.53–0.82)	0.02
IONIZED CALCIUM (MM)	1.25 ± 0.04(1.18–1.33)	1.25 ± 0.04(1.16–1.30)	NS
INTACT PTH (PG/ML)	26.2 ± 8.9(13–40.6)	24 ± 8.2(12–40.7)	NS
PHOSPHATE (MM)	1 ± 0.14(0.58–1.3)	1.06 ± 0.2(0.7–1.7)	NS

Data are means ± SD (range).
*Control group vs. diabetic group.

diabetic and control samples to avoid interassay variations. Intraassay CV was 3.6%. Blood chemistries were measured with routine laboratory methods (Hitachi 737 Autoanalyzer Boehringer Mannheim, Mannheim, Germany) . We determined HbA_{1c} with automated HPLC (Auto A_{1c} HA-8110, Menarini, Barcelona, Spain). Ionized calcium, intact PTH, and magnesium were measured, respectively, with an ion-selective analyzer (Nova 7, Nova Biomedical, Waltham, MA), IRMA (Nichols Instrument Kit, Nichols, San Juan Capistrano, CA), and flame atomic absorptiometry.

Statistical analysis

Results, expressed as means ± SD and range, were compared with the Mann-Whitney *U* test and considered significantly different if $P < 0.05$. For correlation we used *r* coefficient.

RESULTS — Glycemia and HbA_{1c} were increased in the diabetic patients ($P < 0.0001$), whereas magnesium levels were decreased ($P = 0.02$), as shown in Table 1. Intraplatelet cGMP levels were 0.32 ± 0.16 pmol/10^9 platelets (range 0.10–0.64) in the IDDM group versus 0.52 ± 0.32 pmol/10^9 platelets (range 0.13–1.31) in the control group ($P = 0.032$). Plasmatic cGMP levels were no different between groups (0.98 ± 0.43 [range 0.61–2.6] vs. 1.11 ± 0.5 nM [range 0.57–2.65], respectively, $P = 0.6$). Intraplatelet cGMP levels correlated negatively with the duration of the disease ($r = -0.43$, $P < 0.05$), but they did not correlate with the other clinical or biochemical parameters studied.

CONCLUSIONS — This study demonstrates that intraplatelet cGMP levels are decreased in IDDM patients and provides another mechanism to explain the increased platelet aggregability in diabetes. Because cGMP also decreases platelet adhesion (2,3), the low cGMP levels may be partly responsible for increased adhesiveness in diabetes (1). Intraplatelet cGMP levels represent the balance between the activity of the nitric oxide–responsive soluble guanylate cyclase (synthesis) and that of cGMP-PDE (catabolism).

Platelets have ANP receptors, but ANP's occupancy of these sites results in minor changes in the production of intraplatelet cGMP (2). Therefore, the most important factor in the platelet synthesis of cGMP is nitric oxide. On the other hand, plasmatic cGMP levels are strongly influenced by ANP (7). We found normal levels of plasmatic cGMP in diabetes, in accordance with previous reports (8).

The low intraplatelet cGMP levels in diabetes could be attributable to different reasons: *1*) decreased release of nitric oxide from the vascular endothelium and/or decreased intraplatelet synthesis of endogenous nitric oxide; 2) quenching of nitric oxide by advanced glycosylation products; 3) subnormal guanylate cyclase activity; or 4) increased cGMP-PDE activity. An increase in the activity of soluble cGMP-PDE has been found in spontaneously diabetic rats, but in STZ-induced diabetic rats this activity is reduced (9). Further studies are necessary to clarify this issue.

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