The GTP-Binding Regulatory Proteins, Gs and Gi, Are Altered in Erythrocyte Membranes of Patients With Ischemic Heart Disease Resulting From Coronary Atherosclerosis


Acute ischemic heart disease is associated with alterations in the cardiac adenylate cyclase system response, although the specificity and mechanism of these events are unknown. We studied the characteristics of inhibitory (Gi) and stimulatory (Gs) GTP-binding regulatory proteins (G proteins) of adenylate cyclase in erythrocyte membranes of patients (n=16) with nonacute ischemic heart disease resulting from coronary atherosclerosis. Gi was measured by reconstitution with the resolved catalytic unit of adenylate cyclase and by cholera toxin-catalyzed ADP-ribosylation of a 42-kD protein; Gs was tested as a 41-kD substrate of pertussis toxin-catalyzed ADP-ribosylation. Gi activity was decreased by 27±2% in the cholate extract and by 25±3% in the supernatant of guanosine 5'-[(y-thio)triphosphate]-treated membranes. The amount of cholera toxin substrate was decreased by 33±3%, and the pertussis toxin substrate was increased by 27±5% compared with healthy subjects (n=10). All changes in G-protein characteristics appear to be specific relative to other erythrocyte membrane proteins and hemoglobin. Those patients who have a decreased Gi possess approximately normal Gs, and those with increased Gs showed no change in Gi. Patients with increased Gi (normal Gs) exhibited more severe deterioration of their coronary arteries than did patients with decreased Gi (normal Gs) (P<.05), but these two groups did not differ significantly in serum lipids, hormones, drug therapy, historical data, or baseline assessment (P<.05). (Arteriosclerosis and Thrombosis 1993;13:1244-1251)

KEY WORDS • coronary artery disease • ischemic heart disease • human erythrocyte membranes • GTP-binding proteins • pertussis and cholera toxin-catalyzed ADP-ribosylation • adenylate cyclase
tion, which represent, with some limitations, the amounts of $G_i$ and $G_i$, respectively, in erythrocyte membranes of patients with nonacute ischemic heart disease resulting from coronary atherosclerosis. Because samples of heart tissue are not available from patients with the nonacute stage of disease because no heart transplantation or other surgical interventions are required, the only way to determine $G$-protein characteristics is by the study of blood cells. We selected erythrocytes for the reasons listed below. First, human erythrocyte membranes are easily available, so a large enough group of patients may be investigated. Second, the membrane preparation is pure and contains only plasma membranes. Third, human erythrocytes lack adrenergic receptors as well as functional adenylate cyclase activity, which are both lost during reticulocyte maturation, so that the effect of catecholamines on the steady-state levels of $G$ proteins does not occur. Fourth, the methods of determination of $G$-protein activity and level in erythrocyte membranes are well defined, and they are even used as a standard. Fifth, we selected patients without severe biochemical abnormalities with the exception of coronary artery disease and avoiding including patients with acute ischemia; this enabled us to determine the alterations in $G$-protein characteristics that occur before the onset of serious metabolic disorders, since all patients were in the initial stages of the pathological process.

**Methods**

**Patients**

The study comprised 16 male patients with ischemic heart disease aged 35 to 58 years (mean, 47 years). All subjects were under treatment at the Research Center for Preventive Medicine and gave written permission to participate in the study. Fourteen patients had angina pectoris functional class I through III, and 2 had chest pain syndrome due to neurocirculatory asthenia. Eight patients had concomitant essential mild hypertension; plasma cholesterol levels did not exceed 260 mg/dL (mean±SEM, 238±12 mg/dL), which indicates the absence of significant hypercholesterolemia in the Moscow population. Patients with diabetes mellitus, thyroid disease, and previous myocardial infarction were withdrawn from the study. To verify the presence of coronary atherosclerosis and with the aim of choosing further drug therapy, coronary bypass surgery, or percutaneous transluminal coronary angioplasty, all patients had undergone coronary arteriography under intracoronary injection of nitroglycerin (200 μg per each artery) and demonstrated significant (more than 50%) narrowing of at least one coronary artery. Films were recorded using Cardaskop-U angiography apparatus (Siemens) equipped with a K-30 video camera. The image was translated to the Matrix Videotron-LFP/640 system (Matrix Computer AG), which combines the Matrix VME data and the Leutron digital image-processing system. Artery segments (eight to 12) were manually outlined three times each using original software, and the mean diameter value was used for further calculations. Interobserver and intraobserver variations were 3% and 7%, respectively. Global coronary atherosclerosis score (GCS), an index showing the severity of coronary atherosclerosis, was estimated by two observers independently as follows. First, the percent stenosis ($\%S$) was calculated according to the equation

$$\%S = \left(1 - \frac{2D_{\text{max}}}{D_{\text{pra}} + D_{\text{diam}}}\right) \times 100$$

where $D_{\text{max}}$, $D_{\text{pra}}$, and $D_{\text{diam}}$ are minimal, proximal, and distal diameters of arteries, respectively. Second, the degree of stenosis (DS) was estimated: DS was assumed to be 0 when no occlusion was detected; 1, if $\%S$ ranged from 1% to 25%; 2, if $\%S$ ranged from 26% to 50%; 3, if $\%S$ was 51% to 70%; 4, if $\%S$ was 71% to 90%; 5, if $\%S$ was 91% to 99%; and 6, for complete occlusion. Localization of stenosis (LS) was assumed to be 1 for all arteries after ramification; 2, for the distal; 3, for the middle; and 4, for the proximal part of arteries. GCS was calculated according to the equation

$$\text{GCS} = \frac{\Sigma (\text{DS} \cdot \text{LS})}{5}$$

The exercise tests were conducted on a bicycle ergometer produced by Elema. A common technique of graded exercise was used. The starting point was 50 W, with subsequent incremental increases of 25 W. Every exercise stage lasted 3 minutes.

Patients received nitrates (isosorbide dinitrate, 40 to 80 mg/d), calcium antagonists (verapamil, 240 mg/d), $\beta$-blockers (propranolol, 40 to 80 mg/d), and aspirin (0.1 g/d).

The control group comprised 10 healthy subjects (male, aged 27 to 46 years; mean, 38 years) without complaints. All volunteers had normal blood pressure and serum cholesterol levels (less than 220 mg/dL; mean±SEM, 186±8 mg/dL) and showed no abnormalities on the electrocardiogram.

**Preparation of Erythrocyte Membranes**

A venous blood sample was withdrawn by a standard Vacutainer at 8 AM after a 14-hour fast. EDTA was used as an anticoagulant. Erythrocytes were isolated using the Ficoll-Paque method as described by the instructions of the manufacturer (Pharmacia). Cells were removed from the bottom of the tubes and the hemoglobin content per 10^12 cells was determined. No contamination with lymphocytes was observed. Two milliliters of erythrocytes was washed three times in 10 mL of an isotonic solution containing 5 mmol/L sodium phosphate, pH 7.8, and 140 mmol/L NaCl. Membranes were prepared by the method of Dodge et al with modifications. The final membrane preparation (10 mg of protein per milliliter) was divided into small aliquots, frozen in liquid nitrogen, and stored at $-70^\circ$ for 2 months. Such storage conditions did not result in any detectable loss of $G_i$ activity or pertussis and cholera toxin–catalyzed ADP-ribosylation, as determined in a separate experiment. The preparation and storage procedures were standardized for all samples to avoid any possible differences. On the day of the experiment, membranes were thawed and washed twice with 5 volumes of TED buffer containing 20 mmol/L tris(hydroxymethyl)aminomethane (Tris) HCl, pH 8.0, 1 mmol/L EDTA, and 1 mmol/L dithiothreitol (DTT).
Assay of G\textsubscript{i} Activity

G\textsubscript{i} activity was measured by reconstitution with the resolved catalytic unit of adenylate cyclase in phospholipid vesicles. The catalytic unit of adenylate cyclase was partially purified from bovine brain caudate nuclei and resolved from G\textsubscript{i} by the method of Neer and Salter, and reconstitution was performed as described previously. G\textsubscript{i} activity was assayed in two ways: (1) For "total" G\textsubscript{i}, erythrocyte membranes were resuspended in an initial volume of TED buffer containing 1% sodium cholate and 600 mmol/L NaCl, stirred for 1 hour at 4°C, and centrifuged at 100,000g for 1 hour. The supernatant containing G\textsubscript{i} was diluted with 3 volumes of TED buffer; guanosine 5'-[γ-thio]triphosphate (GTP\textsubscript{γ}S) and MgCl\textsubscript{2} were added to make their final concentrations 0.1 mmol/L and 10 mmol/L, respectively. Samples were incubated for 1 hour at 30°C and thus, activated cholate extract was immediately reconstituted with the adenylate cyclase preparation. (2) For "soluble" G\textsubscript{i}, we have previously shown that human erythrocyte G\textsubscript{i} dissociates specifically from membranes in the presence of nonhydrolyzable GTP analogues. Membranes were resuspended in the initial volume and incubated in TED buffer containing 0.1 mmol/L GTP\textsubscript{γ}S and 10 mmol/L MgCl\textsubscript{2} for 3 hours at 30°C. Samples were centrifuged at 30,000g for 40 minutes and the supernatant was reconstituted with adenylate cyclase within 1 hour.

The final assay volume was 50 μL. Fifteen microliters (50 μg of protein) of the adenylate cyclase preparation containing 0.4 mol/L (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} was mixed with either 10 μL of activated, diluted cholate extract for "total" G\textsubscript{i} (2 to 5 μg of protein) or 10 μL of the supernatant of GTP\textsubscript{γ}S-treated membranes for "soluble" G\textsubscript{i} (0.2 to 1 μg of protein) and incubated for 30 minutes at 30°C. 25 μL of incubation mixture containing 100 mol/L Tris-HCl, pH 8.0, 10 mmol/L 3-isobutyl-1-methylxanthine, 4 mmol/L cAMP, 0.2 mmol/L ATP (0.25 μCi of [\textsuperscript{32}P]ATP per sample), 20 mmol/L MgCl\textsubscript{2}, 200 μmol/L GTP\textsubscript{γ}S, 0.8 mg/mL creatine kinase, 40 mmol/L creatine phosphate, 2 mmol/L EDTA, and 10 mmol/L DTT were then added. Incubation was carried out for 1 hour at 37°C and the amount of [\textsuperscript{32}P]cAMP produced was estimated as described. All samples were assayed simultaneously on the same day using the same preparation of catalytic unit in duplicate, and the experiments were repeated three times. The background activity (boiled, G\textsubscript{i}-containing fractions or those reconstituted in the absence of GTP\textsubscript{γ}S and MgCl\textsubscript{2}, gave the same results) was subtracted from the obtained value, and activity was expressed in picomoles of cAMP produced per minute per milligram of either initial cholate extract ("total" G\textsubscript{i}) or total membrane protein ("soluble" G\textsubscript{i}).

The assays were linear for the indicated amount of protein and the indicated incubation time. Because of the relatively high interassay variation (7±4%), three different experiments were analyzed independently. Results were expressed as the percent activity of patient samples vs the G\textsubscript{i} activity of healthy donor samples (assumed to be 100%).

Cholera and Pertussis Toxin-Catalyzed [\textsuperscript{32}P]ADP-Ribosylation

Erythrocyte membranes were resuspended in half of the initial volume and subjected to ADP ribosylation. The incubation mixture for cholera toxin labeling in a final volume of 40 μL contained 250 mmol/L potassium phosphate, pH 6.8, 1 mmol/L EDTA, 20 mmol/L thymidine, 1 mmol/L ATP, 0.1 mmol/L GTP, 10 μmol/L NAD (5 μCi of [\textsuperscript{32}P]NAD per tube), 1 mmol/L NADP, 0.1 mg/mL of cholera toxin (activated in the presence of 20 mmol/L DTT, 0.1% bovine serum albumin, and 0.1% sodium dodecyl sulfate [SDS] for 30 minutes at 30°C), 10 μg of guanyl-5′-imidodiphosphate–treated human erythrocyte cytosol, and 10 μL of membrane protein (0.2 to 0.9 mg/mL). We followed the recommendations of Rosenthal et al and added NADP to inhibit endogenous NADase; NADase activity of the membrane preparation was undetectable under these assay conditions. Incubation was carried out for 1 hour at 30°C. The reaction was stopped by addition of 15 μL of a denaturation mixture and boiling of the samples for 3 minutes. Boiled samples were analyzed by SDS–polyacrylamide gel electrophoresis (PAGE). We avoided centrifugation of the samples before solubilization because the α subunit of G\textsubscript{i} is released from the membrane after cholera toxin–catalyzed ADP-ribosylation, thus decreasing and underestimating the observed extent of ADP-ribosylation.

The incubation mixture for pertussis toxin labeling in the final volume of 40 μL contained 50 mmol/L Tris-HCl, pH 7.8, 10 mmol/L DTT, 2 mmol/L MgCl\textsubscript{2}, 1 mmol/L EDTA, 1 mmol/L NADP, 20 mmol/L thymidine, 1 mmol/L ATP, 0.1 mmol/L GTP, 10 μmol/L NAD (2 μCi of [\textsuperscript{32}P]NAD per tube), 0.15% Lubrol PX, 20 μg/mL of pertussis toxin (activated in the presence of 20 mmol/L DTT, 0.1% Lubrol PX, and 1 mmol/L ATP for 30 minutes at 37°C), and 5 μL of membrane protein (0.1 to 0.45 mg/mL). The addition of Lubrol PX was necessary because in the absence of detergent, pertussis toxin ADP-ribosylates only 5% to 10% of the total G\textsubscript{i}. Incubation was carried out for 1 hour at 37°C and the reaction was stopped by addition of 15 μL of a denaturation mixture and boiling for 3 minutes. Boiled samples were analyzed by SDS-PAGE. G\textsubscript{i} protein, purified from bovine brain, served as a reference standard. Addition of βγ subunits had no effect (n=4, P<.05) on pertussis toxin–catalyzed modification under our experimental conditions.

In general, all samples were assayed simultaneously in duplicate and the experiments were repeated three times with the same results. Both assays were linear within the protein concentrations indicated. The correlation coefficients calculated for three different membrane preparations as described below were 0.96±0.07 for the cholera toxin–catalyzed reaction and 0.98±0.08 for the pertussis toxin–catalyzed reaction. The extent of both toxin-catalyzed ADP-ribosylation reactions reached a stable (up to 1.5 hours) plateau after 45 minutes of incubation as determined in a separate experiment. The addition of fresh toxins after 45 minutes of incubation had no effect on label incorporation.

In the case of cholera toxin the labeling of a 42-kD protein (G\textsubscript{il}α) was estimated, and in the case of pertussis toxin the labeling of a 41-kD protein (G\textsubscript{al} plus G\textsubscript{a2}) was measured (Figure). Human erythrocyte membranes possess only one form of G\textsubscript{i}α and three forms of G\textsubscript{a}, (G\textsubscript{il}α, 41 kD; G\textsubscript{al2}, 41 kD; and G\textsubscript{al3}, 43 kD). The ADP-ribosylation of G\textsubscript{a3}α is low and does not correlate with the amount of protein in membranes.
ADP-ribosylation of G proteins by cholera and pertussis toxins as revealed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Lanes 1 and 2, erythrocyte membrane proteins (35 and 90 μg, respectively) resolved by SDS-PAGE, gels stained for protein using Coomassie Brilliant Blue R-250. Lanes 3 through 7 show autoradiograms of gels loaded with equal amounts of protein (45 μg), which have been treated with pertussis (lanes 3 and 4) or cholera (lanes 5 and 6) toxin or without toxin (lane 7). Gels were exposed to x-ray film for 22 hours (lanes 3 and 5) or 44 hours (lanes 4, 6, and 7). Numbers at left indicate molecular mass markers in kilodaltons. The positions of Gαi and Gαs are indicated on the right. It is evident that addition of toxins is absolutely required for ADP-ribosylation because no label incorporation occurs (lane 7) in the absence of toxins.

**SDS-PAGE and Quantification of Results**

Polyacrylamide gels (10%, 1 mm thick) were run in the system of Laemmli in the presence of 0.1% SDS at a constant current of 25 mA for 1.5 hours at room temperature. Gels were fixed for 30 minutes in 25% 2-propanol and 10% acetic acid; stained for 30 minutes in 50% ethanol, 10% acetic acid, and 0.5% Coomassie Brilliant Blue R-250; destained for 10 minutes in boiling water; dried for 45 minutes; and autoradiographed for 22 and 44 hours in an intensifying screen cassette with Amersham Hyperfilm MP (preflashed). A typical example of the ADP-ribosylation assays is presented in the Figure. Both films (22 and 44 hours) were scanned with an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane.

The average value for each lane was calculated. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ultroscan XL laser densitometer (LKB) three times for each lane. The densitometer was linked to an Ul...
Table 1. G, Activity and Cholera and Pertussis Toxin-Catalyzed ADP-Ribosylation Substrates in Patients and Healthy Donors

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Healthy donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Soluble&quot; G,</td>
<td>75±3</td>
<td>100±6</td>
</tr>
<tr>
<td>&quot;Total&quot; G,</td>
<td>73±2</td>
<td>100±4</td>
</tr>
<tr>
<td>Cholera toxin substrate</td>
<td>67±3</td>
<td>100±5</td>
</tr>
<tr>
<td>Pertussis toxin substrate</td>
<td>127±5</td>
<td>100±3</td>
</tr>
</tbody>
</table>

*Absolute values for G, activity and cholera and pertussis toxin substrates varied between different experiments, so the normalized results are presented (patients vs healthy donors, whose values were assumed to be 100% in each separate experiment) as the mean±SEM from three independent experiments run in duplicate. The absolute values were 40 to 47 pmol cAMP/min per milligram of membrane protein for "soluble" G,; 31 to 36 pmol cAMP/min per milligram of protein in cholate extract for total G,; 0.02 to 0.04 arbitrary units for the 22-hour and 0.04 to 0.08 arbitrary units for the 44-hour autoradiogram for the cholera toxin substrate; 0.06 to 0.09 arbitrary units for the 22-hour and 0.12 to 0.18 arbitrary units for the 44-hour autoradiogram for the pertussis toxin substrate. All G-protein parameters of patients and healthy donors were significantly different at P<.05 as determined by the rank test.

Table 2. Proteins in Erythrocyte Membranes of Healthy Donors and Patients*

<table>
<thead>
<tr>
<th>Band†</th>
<th>Relative mass fraction (% of total membrane protein)</th>
<th>Patients</th>
<th>Healthy donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 and 2</td>
<td>27±2</td>
<td>28±3</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>4.6±0.7</td>
<td>4.6±0.6</td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>5.2±0.4</td>
<td>5.5±0.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4.3±0.2</td>
<td>4.1±0.5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5.5±0.8</td>
<td>4.8±0.8</td>
<td></td>
</tr>
</tbody>
</table>

*The amount of protein was estimated as described in "Methods" by laser densitometry.
†Band names were according to Fairbanks et al. All values were not significantly different as determined by the rank test.

observed. In contrast, the amount of pertussis toxin substrate, representing the G,α subunit, was increased by 27% (Table 1). These changes appear to be specific because no other erythrocyte protein relative-mass fractions were altered. Table 2 contains a list of the five major protein bands. This was also true for up to 17 minor protein bands. These data together with the hemoglobin measurements (4.9±0.2 g/10^12 cells in healthy donor samples and 4.9±0.3 g/10^12 cells in patient samples) indicate that erythrocytes from both healthy donors and patients were indistinguishable in their hemoglobin content and membrane composition.

To elucidate the possible mechanism of G-protein alterations, we analyzed the G-protein characteristics of each patient individually. Individual analysis revealed a very high correlation between "total" G, "soluble" G, and the amount of cholera toxin substrate (r=0.92±0.06, P<.01), thus enabling us to combine these characteristics as one G,α. Moreover, the patients were observed to have either an increased G, or a decreased G,α, i.e., these events did not occur simultaneously. This fact allowed us to split the initial group of patients into two smaller groups and to analyze them independently. One group contained patients with an increased G, but a normal level and activity of G,α, and the other group had a decreased G,α but normal or insignificantly increased (by means of exclusion Q criteria, P<.05) G, (Table 3). It is evident that such differences, although they would result in the alteration of adenylate cyclase in both groups, may be due to either increased inhibition of adenylate cyclase activity (group 2, with increased G,) or decreased stimulation of adenylate cyclase (group 1, with decreased G,).

This finding suggests a marked difference between these two groups, but we were not able to detect them on the basis of other factors. These groups were not significantly different with respect to lipid levels (Table 3), hemoglobin content, plasma concentrations of various hormones (Table 4), drug therapy, historical data, and baseline clinical assessment (Table 5). The only difference detected was that in GCS, an index showing the status of coronary atherosclerosis. The patients who exhibited more severe atherosclerosis (GCS of 15.0±1.8) represent the group with an increased G,α, and those with milder forms of coronary artery disease (GCS of 8.4±1.2) represent the group with a decreased G,α. When the correlation between GCS and G proteins was investigated, these findings became evident, since there was a positive correlation between GCS and G, ADP-ribosylation and a negative one between GCS and G, characteristics, although they were not strong enough to be significant (+0.83±0.12 and -0.7±0.18, respectively; P<.1). The correlation between G proteins and the other tested parameters listed in Tables 3, 4, and 5 was significantly lower, with means ranging from -0.44 to 0.57 (data not presented). The same was true when nonlinear (exponential, logarithmic, and hyperbolic) correlations were determined.

### Discussion

The determination of G-protein characteristics in erythrocyte membranes is a well-defined procedure, so human erythrocyte membranes are often used as a standard. We have slightly updated the methods for determination of G, activity by reconstitution with the resolved catalytic unit of adenylate cyclase (SEM was typically 2% to 4%) and for estimation of pertussis (SEM was 4% to 6%) and cholera (SEM did not exceed...
TABLE 4. Serum Hormone Levels of Two Groups of Patients Exhibiting Different Mechanisms of G-Protein Alteration

<table>
<thead>
<tr>
<th></th>
<th>Group 1: decreased Gs, normal G/ (n=9)</th>
<th>Group 2: increased Gs, normal G/ (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenaline (nmol/L)</td>
<td>2.7±1.2</td>
<td>2.4±1.5</td>
</tr>
<tr>
<td>Noradrenaline (nmol/L)</td>
<td>5.5±0.7</td>
<td>5.2±0.3</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>742±6</td>
<td>751±3</td>
</tr>
<tr>
<td>Insulin (mU/mL)</td>
<td>8.0±2.1</td>
<td>9.9±2.5</td>
</tr>
<tr>
<td>T3 (nmol/L)</td>
<td>1.15±0.12</td>
<td>0.97±0.06</td>
</tr>
<tr>
<td>T4 (nmol/L)</td>
<td>84±8</td>
<td>93±5</td>
</tr>
<tr>
<td>Testosterone (nmol/L)</td>
<td>51±7</td>
<td>41±4</td>
</tr>
<tr>
<td>Cortisol-binding globulin (nmol/L)</td>
<td>171±31</td>
<td>177±42</td>
</tr>
<tr>
<td>GH (ng/mL)</td>
<td>11.5±12</td>
<td>11.8±8</td>
</tr>
<tr>
<td>TSH (mU/mL)</td>
<td>2.8±2</td>
<td>2.9±1.7</td>
</tr>
<tr>
<td>FSH (mU/mL)</td>
<td>4.3±0.6</td>
<td>4.7±0.7</td>
</tr>
<tr>
<td>Thyroxine-binding globulin (nmol/L)</td>
<td>7.4±3.2</td>
<td>8.6±7.4</td>
</tr>
<tr>
<td>Testosterone-binding globulin (nmol/L)</td>
<td>21.7±4</td>
<td>24.7±6</td>
</tr>
<tr>
<td>LH (mU/mL)</td>
<td>4.6±0.5</td>
<td>4.7±0.4</td>
</tr>
</tbody>
</table>

T3, triiodothyronine; T4, thyroxine; GH, growth hormone; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

10%) toxin substrates. This allowed us to observe alterations in Gs activity and the quantity of toxin substrates in patients with ischemic heart disease and healthy subjects (Table 1). These alterations appeared to be specific, because the mass fractions of other membrane proteins appeared to be unchanged (Table 2). This is an additional advantage of the experimental design used in the present study because even after short periods of myocardial infarction or during severe heart failure, very significant alterations in heart muscle tissue may occur, thus affecting quantification of the G proteins and adenylate cyclase activity.35

G-protein alterations in general are a result of the processes listed below. A decrease in Gs and an increase in G, in the steady-state levels of endogenously ADP-ribosylated proteins may affect the extent of ADP-ribosylation by cholera and pertussis toxins. Endogenous ADP-ribosyltransferases present in erythrocytes may substantially lower the degree of cholera66 as well as pertussis77 toxin-catalyzed labeling, but they would not affect the reconstitution activity of Gs, thus confirming the decrease in cholera toxin substrate. The increase in pertussis toxin substrate cannot be explained by an increase in endogenous cysteine-specific ADP-ribosylation or phosphorylation of Gax,38 since these events are able to only lower the apparent amount of pertussis toxin substrate. Changes in other membrane proteins and enzymes are able to influence the observed value of ADP-ribosylation. The latter possibility seems not to be true, because the addition of βγ subunits purified from bovine brain did not stimulate ADP-ribosylation of Gs by pertussis or of Gs, by cholera toxin. Moreover, the guanyl-5'-imidodiphosphate–treated erythrocyte cytosol used as a source for ADP-ribosylating factor for cholera toxin–catalyzed ADP-ribosylation59 was added in excess, so the ADP-ribosylating factor did not affect the cholera toxin–catalyzed reaction. More likely is that a change in the amount of protein (Gax and Gsα+G1α) occurred.

The question about the mechanism of such alterations has several answers in general. First, the amount of G proteins may be changed during the reticulocyte stage of maturation under the influence of hormones acting through the cAMP40,41 or other receptor-dependent pathways. Second, differential or impaired selective degradation of G proteins may occur in erythrocytes.33 Third, the proportion of soluble or cytoplasmic G proteins is possibly subject to change,44 but we observed no decrease in the guanine nucleotide–dependent dissociation of Gs (“soluble” Gs) relative to the amount of “total” Gs.

To determine whether G proteins are changed in response to hormonal stimulus or any physiological changes, we determined the correlations between G protein alterations and a number of parameters of lipid levels, oxygen consumption, hormone metabolism, and drug therapy and found no significant correlations. This may be due to the insufficient number of patients investigated. Nevertheless, only one of the tested parameters, GCS, correlated with G-protein characteristics. Then we used the individual approach and noticed that patients have either an increased Gs or a decreased Gs, but not both of these simultaneously. This fact allowed us to split the patients into two groups as characterized in Tables 3, 4, and 5. No difference between these groups was found with one exception: a significant difference in GCS was observed.

G-protein alterations are likely to be ubiquitous in cardiovascular disease. They occur in the heart, muscle, lymphocytes and, as shown in the present study, erythrocytes. Thus, the tissue specificity of G-protein alter-
ations as postulated by Feldman\(^6\) becomes questionable. Functional defects in G\(_\alpha\) and G\(_\alpha\) may contribute to the different abnormalities in cardiovascular pathology. The data of the present study apparently contrast with the findings of Bohm et al,\(^4,5\) who did not detect G, alterations in heart membranes of patients with ischemic cardiomyopathy. However, these authors did not estimate the lesions of coronary arteries in their group of patients; additionally, our patients did not exhibit heart failure (Table 5). Thus, one cannot extrapolate from the results of Bohm et al to G-protein characteristics in ischemic heart disease or moreover to erythrocyte membrane G proteins.

We speculate that G-protein levels are altered independently of functional adenylate cyclase (ie, cAMP), plasma lipids, hormones, drug therapy, and a number of physiological parameters but are correlated with coronary atherosclerosis lesion status. No function for either G, or G, can be definitively stated for human red blood cells because of their lack of functional adenylate cyclase activity and \(\beta\)-adrenergic receptors, although they may interact with other receptors, effector enzymes,\(^1\) and Na\(^+,\)K\(^-\)ATPase.\(^6,66\) Recently, Buhler et al\(^47\) suggested that low-density lipoprotein and high-density lipoprotein regulate the interactions of many cell types, acting through their receptors in a manner similar to other hormones, and that these receptors may be coupled to G proteins. Hence, G-protein level alterations are able to change cellular responses to lipoprotein effects and thus contribute to atherogenesis.

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References


The GTP-binding regulatory proteins, Gs and G(i), are altered in erythrocyte membranes of patients with ischemic heart disease resulting from coronary atherosclerosis.

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