Immunoenzymometric Assay of Human Glycogen Phosphorylase Isoenzyme BB in Diagnosis of Ischemic Myocardial Injury

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With a new immunoenzymometric assay we measured human glycogen phosphorylase isoenzyme BB (GPBB) in 116 healthy individuals, 14 patients with stable angina, 107 nontraumatic chest pain patients on admission to the emergency department [45 acute myocardial infarction (AMI), 49 unstable angina, 13 other diseases], and in serial samples from 41 AMI patients. GPBB was compared with creatine kinase (CK), CKMB mass, myoglobin, and cardiac troponin T. Receiver-operating characteristic plots demonstrated the significantly greater (P = 0.012) discriminatory power of GPBB to detect acute ischemic coronary syndromes compared with all other tested markers. GPBB was the most sensitive marker for detection of AMI during the first 4 h after onset of chest pain, and only GPBB was increased above the upper reference limit (7 µg/L) on admission in patients who had unstable angina at rest and reversible ST-T alterations. This and the high early sensitivity of GPBB are most likely explained by its function as a key enzyme of glycogenolysis.

Indexing Terms: myocardial infarction/unstable angina pectoris/creatine kinase/myoglobin/troponin T

Early identification and confirmation of acute myocardial infarction (AMI) is essential for correct patient care and disposition decisions (1).6 Because as many as one-fourth of AMI patients present with atypical signs and symptoms (1, 2), ~5% of patients with AMI are released unintentionally from the emergency department, which places them at greater risk of morbidity and mortality resulting from complications (2). Although electrocardiography (ECG) is considered the most simple, convenient, reliable, and reproducible method for early diagnosis of AMI, approximately half of all AMI patients have nondiagnostic ECGs at the time of presentation to the emergency department (1, 2). Creatine kinase (CK) and CKMB activities traditionally have not been considered helpful in the emergency evaluation of the patient with chest discomfort (3). The search for more sensitive biochemical markers to indicate acute myocardial ischemia during its early phase (3, 4) reflects the need for an improved initial diagnostic accuracy of these markers in patients with acute chest pain. In addition, biochemical markers may gain increasing importance for the noninvasive monitoring of the effectiveness of thrombolytic treatment (5).

Human glycogen phosphorylase (GP) b (EC 2.4.1.1), particularly its isoenzyme BB (GPBB), has a distinct sensitivity to myocardial oxygen deficiency in cardiomyocytes (6, 7). There is evidence that GPBB is rapidly released into the circulation in the early phase of AMI (8, 9) as well as after periperaoperative myocardial infarction following coronary artery bypass grafting (10, 11). GP in mammals is known to have three major isoenzymes: BB (brain), MM (muscle), and LL (liver) (12, 13). GPBB is also found in heart muscle (14, 15), including human myocardium (16), where it is, besides the MM subtype, the predominant isoenzyme. The three isoenzymes can be distinguished by functional and immunological properties, and are also encoded by three distinct genes (17). The cDNAs encoding human BB, MM, and LL isoenzymes of phosphorylase have been cloned and sequenced (17). The protein sequence deduced from the nucleotide sequence of GPBB cDNA is 83% identical to muscle sequence and 80% identical to liver sequence (17). However, the C-terminal portion of the BB isoenzyme has 21 and 16 additional amino acid residues, respectively, that are not present on the MM and LL isoenzymes, which makes this protein a promising analyte for isoenzyme specificity. Indeed, by using highly purified human BB antigen isolated from brain (17, 18) and heart tissue (8, 16), sensitive immunoassay methods for measuring immunoreactive GPBB have been established (8, 18, 19).

As a key enzyme of glycogenolysis, cardiac GP is a constituent of the particular sarcoplasmic reticulum (SR) glycogenolytic complex in the cardiomyocytes (20, 21). The degree of association of GP with this

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4 Nonstandard abbreviations: GP, glycogen phosphorylase; GPBB, glycogen phosphorylase isoenzyme BB; CK, creatine kinase; TnT, cardiac troponin T; AMI, acute myocardial infarction; ECG, electrocardiography; IEMA, immunoenzymometric assay; WHO, World Health Organization; CAD, coronary artery disease; CAG, coronary angiography; mAb, monoclonal antibody; URL, upper reference limit; ROC, receiver-operating characteristic; PBS, phosphate-buffered saline; and SR, sarcoplasmic reticulum.

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complex depends essentially on the metabolic state of the myocardium and seems to be highly sensitive to the ischemia-induced glycogen breakdown (22). After changing from a structurally bound form into a soluble cytosolic form during phosphorolysis, the enzyme may penetrate as a dimer through the altered cell membrane into the extracellular space (6, 7, 23). This metabolic sequence reflects the strongly structural and functional coupling of GPBB to the ischemia-sensitive process of glycogenolysis. Therefore, the specific mechanism of GPBB release suggests some new aspects for the laboratory diagnosis of acute myocardial ischemia in human blood that are different from other markers of myocardial damage, such as myoglobin, CK and its isoenzymes MB and MM, and their isoforms, as well as cardiac troponin-T (cTnT), cardiac troponin-I, and fatty acid-binding protein (4, 24–27).

In the present study, we investigated the diagnostic performance of GPBB in identifying different states of myocardial ischemia in patients with AMI, patients with stable and unstable angina pectoris, and emergency-room patients with chest pain. GPBB was compared with myoglobin, CK and its isoenzyme MB, and cTnT. The protein enzyme concentrations of GPBB in human plasma were measured by a new immunoenzymometric assay (IEMA) based on the use of two different monoclonal antibodies (mAbs) (19, 28). Our results demonstrate a high sensitivity of isophosphorylase BB for diagnosing and monitoring acute myocardial ischemia during its early phase.

Subjects and Methods

The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised 1983.

Subjects

Healthy individuals. GPBB was measured in the plasma of 40 apparently healthy blood donors, 46 coworkers, and 30 healthy individuals seen for routine medical examination. This reference population comprised 59 men and 57 women, ages 20 to 66 years (mean 40 years). A single blood sample was drawn from each subject.

Chronic stable angina pectoris patients. This group comprised 14 patients [11 men, 3 women, age 54 ± 9 years (mean ± SD); range 47–64 years] with chronic stable angina who underwent percutaneous coronary angioplasty. A single blood sample was obtained before the angioplasty.

AMI patients. The object of this part of our study was to identify existing differences in the time courses of GPBB and other biochemical markers in plasma after AMI. We investigated 41 patients with AMI (30 men, 11 women, mean age 59 ± 11 years; range 36–79 years) who gave informed consent for extra blood samples to be drawn. World Health Organization (WHO) criteria were followed for the diagnosis of AMI (29, 30). On average, patients were admitted to the coronary care unit 5 h (range 0.5–27.5 h) after the onset of chest pain. Peripheral venous blood samples were collected from an indwelling catheter or by venipuncture before therapy was started in the coronary care unit, hourly for the first 10 h, then every 4 h up to 24 h after admission to the hospital; in 8-h intervals for the subsequent 24 h; and then daily until the patient’s discharge. Patient care requirements occasionally prevented the taking of a sample. Twenty-one patients sustained an anterior and 20 an inferior wall AMI (32 Q-wave and 9 non-Q-wave AMI). All but one patient received intravenous thrombolytic treatment. Eleven patients received streptokinase, 2 received urokinase, 10 were given recombinant tissue-type plasminogen activator, and 17 patients participating in the International Study of Infarct Survival III were randomly assigned to either intravenous streptokinase, the recombinant tissue-type plasminogen activator, or anisoylated plasminogen streptokinase activator complex. Additionally, all patients received routine coronary care and were treated with analgesics, intravenous heparin, acetylsalicylic acid, nitrates, and occasionally beta blockers, furosemide, and antiarrhythmic agents, depending on clinical circumstances.

Acute coronary angiography (CAG) was not routinely performed in the patients investigated. To determine whether there was successful reperfusion of the infarct-related coronary artery in Q-wave AMIs, we used four biochemical criteria that had been evaluated previously by using acute CAG as the criterion standard in AMI patients treated with thrombolytic agents within 6 h after the onset of chest pain (5, 29, 31, 32). In the present study, early reperfusion of the infarct-related artery was assumed when the following biochemical criteria were fulfilled: (a) CK peaks within 12 h; (b) CKMB peaks within 10 h; (c) myoglobin peaks within 3 h; and (d) cTnT peaks within 15 h after administration of thrombolytic treatment (5). In 25 Q-wave AMI patients who were treated within 6 h from the onset of symptoms, data were complete. Of these patients, 18 met these noninvasive criteria for reperfusion and were classified as “early reperfused.” CAG was performed selectively in 18 patients at a mean of 22 days (range 4–44) after AMI.

Nontraumatic chest pain patients. This part of our study evaluated the diagnostic performance of GPBB for the early diagnosis of acute ischemic coronary syndromes defined as AMI and severe unstable angina at rest in emergency room patients with chest pain as their major symptom. The study population of 107 patients (75 men, 32 women; mean age 61 ± 12; range 29–89 years) therefore did not include cases of chest contusion or trauma. The mean delay from the onset of chest pain to admission was 4.7 h (range 0.3–48). Forty-five patients were subsequently determined to have had an AMI (according to WHO criteria), 24 Q-wave and 21 non-Q-wave. Forty-nine patients presented with unstable angina, 17 with severe chest pain and transient ST-T alterations at rest. A further 13 patients had chest pain not related to coronary artery disease (CAD; 2 cases of supraventricular tachycardia, 4 pneumonia with pleurisy, 1 heart failure with pul-
monary edema, 3 musculoskeletal disorders, 1 pulmon-
ary embolism, and 2 perimyocarditis). In these pa-
tients a single blood sample was drawn immediately after admission. All patients received routine emer-
gency treatment. All AMI patients were transferred to
the coronary care unit before initiating thrombolytic
therapy. None of these patients had received throm-
bolitic therapy before admission to the hospital.

Diagnostic Criteria for Classification of Patients

Patients were classified as AMI, unstable angina,
stable angina, and patients without CAD (angina could
be excluded by a definitive alternative source of chest
pain). An acute ischemic coronary syndrome was de-
ned as AMI or severe unstable angina at rest together
with transient ST- or T-wave alterations.

The definite diagnosis of AMI required at least two
of the following three clinical criteria to be positive
(29, 30): (a) typical prolonged severe chest pain and
related symptoms of >20 min duration; (b) the evolu-
tion of abnormal Q waves or equivalents on serial
EKGs in at least two leads of the same vascular
territory; and (c) serial CK and CKMB increases
with an initial rise and a subsequent fall with peak values
of twice the upper limit of the reference interval. An AMI
was called Q-wave myocardial infarction if there were
abnormal Q waves (≥0.04 s, >1/4 R-wave amplitude)
or QS complexes, and non-Q-wave myocardial in-
farction if there was ST-segment depression or an increase
of at least 0.1 mV (limb leads) or 0.2 mV (precordial
leads) and T-wave inversion in at least two leads of the
same vascular territory of at least 24-h duration.

Unstable angina pectoris was diagnosed on the basis
of typical anginal chest pain at rest or brought on by
minimal exertion and (or) a new pattern of chest pain
in patients previously classied as having chronic
angina. Patients were graded according to whether
transient ST-T changes occurred at rest.

Laboratory Analysis

Blood collection. Venous blood was collected in
EDTA-containing tubes (final concentration 4 mmol/L;
Sarstedt, Nürnberg, Germany) and immediately cen-
trifuged at 2000g for 15 min. CK activities were
measured without delay; plasma for measurement of all
other markers was frozen and stored at <−20 °C until
analysis. Not every marker could be measured in all
patients. Therefore, patient cohorts of different num-
bers were used for comparison of markers.

CK. CK activities were measured at 25 °C by means
of an N-acetylcysteine-activated, optimized ultraviolet
test from Merck (Darmstadt, Germany). The upper
reference limits (URLs) of CK are 70 U/L for women
and 80 U/L for men.

CKMB. CKMB mass concentration was measured
by a microparticle enzyme immunoassay (Abbott, Ab-
bott Park, IL) for use with the Abbott IMx automated
analyzer. We previously found an URL of 7 μg/L (25).

Myoglobin. Myoglobin concentration was deter-
mined by a commercially available immunoturbidimet-
ric assay (Behringwerke, Marburg, Germany). The
URL is 70 μg/L (24).

cTnT. cTnT was measured by a prototype enzyme
imunoassay (Boehringer Mannheim, Mannheim,
Germany) that is highly specific for cTnT and was
developed by Katus et al. (26). The URL of this pro-
totype assay in EDTA-plasma samples was 0.5 μg/L (27);
the cross-reactivity with skeletal TnT is reportedly as
high as 3.6% (26).

IEMA for GPBB

Materials. Soluble starch, glycogen for biochemistry,
and o-phenylenediamine were purchased from Merck;
5′-AMP-Sepharose 4B from Pharmacia (Uppsala, Swe-
den); AMP from Boehringer Mannheim; Tween-20,
2-mercaptoethanol, and glycero-2-phosphate from
Serva (Heidelberg, Germany); and polystyrene micro-
titer plates from Nunc (Roskilde, Denmark; Maxisorp)
or from Greiner-Labortechnik (Solingen, Germany;
high binding capacity). EDTA-plasma from healthy
subjects, which was used as zero-plasma for the prep-
paration of controls in IEMA, was purchased from the
public blood donation center in Berlin. Routinely, the
basal concentration of GP was eliminated by affinity
chromatography with 5′-AMP-Sepharose 4B.

Antigen preparation. The b form of GPBB was iso-
lated and purified from human heart muscle tissue as
previously described (16, 33) with slight modifications.
In short, heart muscle tissue (~100 g of material taken
<10 h postmortem at autopsy) was homogenized in
2.5-fold volume of extraction solution (1 mmol/L EDTA,
30 mmol/L 2-mercaptoethanol, 5 mmol/L glycero-2-
phosphate, pH 6.8) and centrifuged at 20 000g at 0 °C.
The supernate was incubated at 37 °C for 45 min; the
protein was precipitated at 4 °C by addition of ammo-
nium sulfate (16); and the pellet was suspended, dia-
lyzed, and subjected to starch gel column chromatog-
raphy (33). The pooled GPBB isoenzyme fraction was
further purified and concentrated by affinity chroma-
tography on 5′-AMP-Sepharose 4B (34). The antigen
was stored in 500 mL/L glycerol at −20 °C. GPBB
enzyme activity was assayed by using an immunoinhi-
bition test previously described by Rabitzsch et al. (8).
The purity of the antigen was evaluated by polyacryl-
amide gel electrophoresis under denaturing conditions
(35) and a nondenaturing system described by Will et
al. (16) for isoenzyme activity detection.

Human skeletal muscle isophosphorylase b (MM)
was isolated with a specific activity of 31 kU/g protein
as previously described (16) with additional affinity
chromatography on 5′-AMP-Sepharose 4B (34). Hu-
mian liver isophosphorylase (LL) was partially purified
according to Appleman et al. (36), resulting in an
activity of 2 kU/L. Polyacrylamide gel electrophoresis
in the presence of sodium dodecyl sulfate presented a
single protein band at 94 kDa. Under nondenaturing
conditions, a single activity band was found in the
presence of 0.5 mol/L sodium sulfate (37).

Production of monoclonal antibodies. mAbs against
GPBB were produced and characterized as previously
described (28, 38). Briefly, female BALB/c-Han mice were immunized by an intraperitoneal injection of 20 \( \mu g \) of GPBB with complete Freund's adjuvant. With Polyethylene Glycol 1500 (Serva) as fusogen, hybridomas were obtained by fusion of myeloma cells Sp2/0 with spleen cells of mice having a serum antibody titer > 1:10 000. Screening of anti-GPBB antibody-producing hybridomas was performed with an immunodot-ELISA previously described by Noll et al. (39). From 576 primary cultures, after three cloning steps, seven hybridoma clones were obtained as stable antibody producers and could be multiplied in vitro (roller culture) as well as in vivo by the ascites method for the mass production of mAbs. The immunoglobulin class specificity of the mAbs obtained was characterized in an immunodot-ELISA with rabbit anti-mouse immunoglobulin isotype-specific antibodies (ICN ImmunobiologicaIs, Lisle, IL). The cross-reactivity of these seven mAbs with the other isoenzymes of GP was tested by immunodot-ELISA (39). Briefly, a well-defined amount of antigen (0.2 \( \mu L \) of an antigen-containing solution (0.15 g/L) adsorbed to nitrocellulose was incubated with excessive amounts of single antibodies (50 \( \mu L \), mAb concentration 50 \( \mu g/L \)). A second enzyme-labeled antibody was used to detect antibody-antigen complexes. According to lack of cross-reactivity (no cross-reactivity of the selected mAbs with isoenzymes MM and LL could be detected) and the epitope analysis with the fluoroscein isothiocyanate (FITC) technique (40), the combination of mAb VID12 (fixed to the solid phase) and mAb IIP11 (labeled with horseradish peroxidase) was selected for the development of the one-step IEMA for GPBB.

**IEMA.** The IEMA for GPBB was performed on microtiter plates. mAb1 was diluted in a solution containing 20 mmol/L potassium hydrogen phosphate, 20 mmol/L potassium dihydrogen phosphate, and 150 mmol/L sodium chloride (PBS, pH 7.3). Peroxidase-conjugated mAb2 was diluted in PBS containing 0.5 mmol/L Tween 20 and 100 mmol/L calf serum. For coating, 100 \( \mu L \) well of mAb2 (7.5 mg/L) was added to polystyrene microtiter plates and incubated at 37 \( ^\circ \)C for 16–18 h. All following steps of the assay were performed at room temperature. The dried plates were washed three times with washing solution [0.5 mmol/L Tween 20 in water (Tw-water)] and again filled with 200 \( \mu L \) well of blocking solution (5 g/L bovine serum albumin, 0.5 mmol/L Tween 20 in PBS) for blocking nonspecific protein binding sites. After shaking for 1 h, plates were emptied and washed three times with Tw-water. Then 50 \( \mu L \) of human zero-plasma as blank (n = 4; see Materials), eight different calibrator samples in duplicate containing 1.25–160 \( \mu g/L \) GPBB in zero-plasma, and plasma samples of patients in duplicate were incubated with shaking in darkness for 90 min together with 50 \( \mu L \) of peroxidase-conjugated mAb2 (0.2 \( \mu g/L \)). After washing the plates more than five times with Tw-water to remove the unbound free conjugate, the peroxidase reaction was started by adding to each emptied well 200 \( \mu L \) of the substrate chromogen solution (0.4 g/L o-phenylenediamine and 0.015% hydrogen peroxide in 70 mmol/L citric acid and 100 mmol/L sodium phosphate, pH 5.0). The reaction was stopped after 15 min by addition of 50 \( \mu L \) of 2 mol/L sulfuric acid, and the absorbance was measured at 492 nm in a microplate reader (HT-II; Anthos, Köln, Germany). GPBB values were calculated as micrograms of isoenzyme protein per liter of blood plasma.

**Assay evaluation.** To validate the IEMA, we performed dilution assays, determined GPBB recovery, examined assay reproducibility, and determined cross-reactivities with MM and LL isoenzymes by adding up to 100-fold greater concentrations of GPMM and GP LL in comparison with GPBB, i.e., plasma concentrations of 160–10 000 \( \mu g/L \).

**Data Analysis**

Data are given as mean ± SD or as median and interquartile range. Analysis of variance and Student's \( t \) and Mann–Whitney tests were used to identify groups differing significantly from each other. \( P \) values <0.05 were considered statistically significant. All tests were performed two-tailed. The 95% confidence intervals were calculated. Qualitative data were analyzed by 2 \( \times \) 2 contingency table analysis and when needed by \( \chi^2 \) test with Yates correction for continuity and Fisher's exact test. Two continuous variables were compared by correlation (Pearson correlation coefficient) and linear regression analysis. For comparison of the different markers, data are given as multiples of their URL. The URL of GPBB was calculated nonparametrically from its concentrations in various reference populations (healthy subjects, patients with chronic stable CAD, and hospitalized nontraumatic chest pain patients without acute ischemic coronary syndromes).

To determine the clinical performance of GPBB for acute ischemic coronary syndromes, we constructed from data of emergency-room patients at admission diagnostic sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio [sensitivity/(1 - specificity)], negative likelihood ratio [(1 - sensitivity)/specificity], and receiver-operating characteristics (ROC) curves. Parametric ROC curves, areas under ROC curves, and the significance of differences between the areas under the curves were calculated with the programs Clabroc and Labroc 1 from C.E. Metz (Department of Radiology, University of Chicago, Chicago, IL) (41). Areas under two ROC curves were compared by an univariate \( z \)-score test.

**Results**

**Isolation of GPBB**

The purified GPBB from human heart in the \( b \) form exhibited a specific activity of 30 kU/g protein. Of the total enzyme activity, >99% GPBB was obtained according to the immunoinhibition assay of the isoenzyme's activity (8). On polyacrylamide slab gels, the purified GPBB presented a single band with detection of enzyme activity in the nondenaturing system (16).
and also after Coomassie Blue staining in the sodium dodecyl sulfate system of Laemmli (35) (data not shown).

Characterization of mAbs

Table 1 shows some of the characteristics of mAbs against GPBB produced by seven different hybridoma clones obtained from one fusion experiment. All mAbs belong to the immunoglobulin class IgG1/κ, except mAb IG12, which is an IgG2a/κ. The specificities of the mAbs regarding cross-reactivities with the isoenzymes MM and LL were tested by immunodot-ELISA with the antigen adsorbed to nitrocellulose. None of the antibodies revealed any cross-reactivity in this assay. Some of the antibodies interfered with the enzyme activity of GPBB. The strongest inhibition was caused by the mAb IIID9 (see Table 1). Thus the recognized epitope should be located at or near the catalytic site of the GPBB molecule.

The screening of all possible mAb combinations by IEMA (the first mAb was adsorbed onto microtiter plates, the second one was fluorescein isothiocyanate-labeled) permitted the use of seven purified mAbs (49 different pairs) to define relative epitopes recognized by the corresponding mAb. As demonstrated in the last column of Table 1, five different antigenic determinants could be defined. Three of the mAbs (IG12, IIA2, and VD4) bind to the same epitope.

Assay Characteristics

The one-step sandwich IEMA of GPBB in human EDTA-plasma was performed by simultaneous incubation of both the immobilized mAb VID12 and the peroxidase-conjugated indicator mAb IIF11.

Measuring range and specificity. The assay enables a specific and sensitive estimation of GPBB in the diagnostically relevant range of 0.5–200 µg/L isoenzyme protein in EDTA-plasma. The lower detection limit of the assay was 0.3 µg/L (3 SD above the mean nonspecific binding of the reagent blank). The calibration curve of GPBB in human EDTA plasma, given in Fig. 1, is linear throughout the total range of 0.625 to 160 µg/L. The degree of cross-reactivity of the skeletal muscle (MM) and liver (LL) isophosphorylases with the

Fig. 1. IEMA calibration curve of GPBB mass (BB) in human EDTA-plasma and cross-reactivities of human liver (LL; △) and skeletal muscle (MM; ○) isophosphorylases.

GPBB IEMA was investigated in plasma concentrations of 160–10 000 µg/L by addition of up to 100-fold excess concentrations of GPMM and GPLL compared with GPBB. On the basis of the sensitivity of the IEMA for GPBB (0.3 µg/L), both isoenzymes showed a cross-reactivity of <1% only (see Fig. 1B), allowing the determination quantitatively of GPBB even in the presence of the other isoenzymes without any substantial interference. Additionally, no cross-reactivity of the selected mAbs with isoenzymes MM and LL could be detected in the immunodot-ELISA (39) (see Subjects and Methods).

Linearity and recovery. The analytical recovery was tested in a range of 15–120 µg/L by addition of GPBB purified from human heart to human zero-plasma. We found a recovery of 105.5% ± 1.92% (mean ± SD; n = 10). The linearity was estimated by 2-, 4-, 8-, and 16-fold dilutions of a plasma sample from a patient with a high mass concentration of GPBB with a zero-plasma. The correlation coefficient calculated from expected and measured mass concentrations was r = 0.994 (y = 1.034x + 0.48).

Precision. The within-run and between-run variations of the assay were calculated for a range of concentrations, starting from below the URL (7 µg/L) to very high (2.5, 10, 40, and 160 µg/L). The intraassay CVs were 5.1%, 4.0%, 5.2%, and 4.7% (n = 18), and the interassay CVs were 11.0%, 11.2%, 10.3%, and 6.0% (n = 16), respectively.

URL. In addition to a previous study (42), we report on GPBB measured in healthy individuals. However, we wanted to establish an URL that is also based on GPBB concentrations found in clinically more relevant reference populations (patients with chronic stable CAD or nontraumatic chest pain patients in whom an acute ischemic coronary syndrome was ruled out sub-

### Table 1. Characterization of mAbs against GPBB.

<table>
<thead>
<tr>
<th>Hybridoma</th>
<th>Immunoglobulin class</th>
<th>H-chain</th>
<th>BB</th>
<th>MM</th>
<th>LL</th>
<th>Inhibition of GPBB activity, %</th>
<th>Epitope recognized on GPBB</th>
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<td>IG 12</td>
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<td>IIA2</td>
<td>IgG1</td>
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<tr>
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*All x light chains.  B Assessed by immunodot-ELISA.*
sequently). The nonparametrically calculated 97.5 percentile of GPBB concentrations in 116 healthy subjects was 7.0 μg/L (Fig. 2; outliers were not eliminated for the calculation); the median was 2.3 μg/L, the interquartile range 1.7–2.9 μg/L. All patients with chronic stable angina pectoris had GPBB concentrations <7 μg/L (median 1.7 μg/L, interquartile range 0.9–4.1 μg/L). GPBB peak concentrations found in AMI patients (range 15–1210 μg/L) were markedly above this limit (Q-wave AMI: median 104 μg/L, interquartile range 41.9–170.4 μg/L; non-Q-wave AMI: median 42.0 μg/L, interquartile range 38.0–56.0 μg/L; Fig. 2). In unstable angina without transient ST-T changes at rest, the median GPBB concentration was 3.9 μg/L (interquartile range 2.8–4.5); in patients with ST-T changes at rest, 12.2 μg/L (8.5–19.3). We calculated a frequency distribution (Fig. 3) of GPBB concentrations in 103 patients who were differentiated according to their clinical diagnoses into (a) 45 patients with CADs: stable angina pectoris (n = 14) and unstable angina pectoris without ECG alterations on admission to the emergency room (n = 31); and (b) 58 patients with acute ischemic coronary syndromes: patients with Q-wave infarction (n = 32), non-Q-wave infarction (n = 9), and unstable angina pectoris with transient ST-T alterations at rest on the admission ECG recording (n = 17). All patients without acute ischemic coronary syndromes had plasma GPBB mass concentration <7 μg/L (Fig. 3). The URL of 7 μg/L could be also confirmed by ROC analysis of GPBB concentrations in nontraumatic chest pain patients (see below).

GPBB Time Course in AMI

All biochemical markers were increased in AMI patients. GPBB was increased in all investigated AMI patients from 7 to 13 h after the onset of chest pain (Fig. 4B). Early sensitivities of biochemical markers during the first 12 h after the onset of chest pain are compared in Fig. 4A. For example, 4 h after the onset of chest pain, GPBB was increased in 70%, myoglobin in 43%, cTnT in 33%, and CKMB mass in 56% of patients. The difference between GPBB and cTnT was significant (P = 0.041). Interestingly, although myoglobin was increased in all AMI patients investigated during the first 12 h after the onset of chest pain, we found no time point at which myoglobin was increased in all investigated patients at the same time, because either myoglobin was not yet increased in all patients (early period) or it had already returned to normal in some patients (late period).

Fig. 2. GPBB in the differential diagnosis of different states of myocardial ischemia. Peak plasma concentrations of patients with non-Q- and Q-wave infarction are compared with samples of healthy subjects and patients with stable angina pectoris (AP), unstable AP without ECG changes (UAP–), and unstable AP with ST-T ECG changes (UAP+). The samples for unstable AP were taken on admission to the emergency room. Median values of groups are indicated by solid lines. Significances were calculated with the Mann-Whitney U-test. URL: 7 μg/L (horizontal line).

Fig. 3. GPBB mass concentrations in patients with CAD with and without acute ischemic coronary syndromes.

Frequency distribution of the highest measured plasma concentrations of GPBB in patients with acute ischemic coronary syndromes (acute CAD: unstable angina with ST-T alterations at rest and AMI; n = 58) and patients without acute ischemic coronary syndromes (CAD: stable angina and unstable angina without ST-T alterations at rest; n = 45).

Fig. 4. Comparison of the sensitivities of GPBB (A), myoglobin (C), CKMB mass (+), and cTnT (□) in patients with AMI during the first 12 h after the onset of chest pain (A) and the diagnostic window of GPBB (B).

Values were calculated as percentage of patients with marker concentrations above the URL at various times after onset of chest pain. The range of the 95% confidence interval of GPBB during the first 12 h after the onset of chest pain is indicated as the shaded area (A).
Similar to other biochemical markers, GPBB time courses in Q-wave AMI were significantly (P = 0.005) influenced by early reperfusion of the infarct-related coronary artery (see Fig. 5). The time to peak values of patients with and without early reperfusion differed significantly (5.7 ± 2.0 vs 14.4 ± 2.4 h, P = 0.006).

GPBB vs Other Biochemical Markers in AMI Patients

GPBB mass, myoglobin, cTnT, CKMB mass concentrations, and CK activity of 18 AMI patients are compared in Fig. 6. For this purpose, only AMI patients admitted ≤4 h after the onset of chest pain were included, because after 4 h the probability strongly increases that all four markers are increased already on admission. For better comparison of markers, concentrations are given as multiples of their URL. Criteria describing the time courses of biochemical markers are listed in Table 2. GPBB is the first marker that increases to pathological concentrations in plasma after AMI. The difference between the first appearance of GPBB and all other markers was significant (P <0.05). Myoglobin showed the earliest peak values, which occurred significantly earlier than the peak values of the other markers (P <0.005) except for GPBB. The time to GPBB peak values differed significantly (P <0.05) from the time to peak values of CKMB, cTnT, and CK. Myoglobin was also the first marker that disappeared in plasma after AMI. The differences between myoglobin and the other markers were highly significant (P <0.0005). After AMI, cTnT showed the highest relative increase of peak values compared with URL (Table 2). The differences in the magnitudes of increase between cTnT and myoglobin, CK, and GPBB were significant (P <0.05). GPBB peak values correlated closely with CKMB (r = 0.84, P = 0.0002) and myoglobin (r = 0.93, P = 0.0001) peaks.

We also compared the early sensitivity of biochemical markers in samples from AMI patients that were obtained before initiating thrombolytic treatment. Patients after cardiopulmonary resuscitation or direct-current countershock therapy were also excluded from data analysis. The most striking feature of GPBB is its high early diagnostic sensitivity. During the first 4 h after the onset of chest pain, GPBB was significantly (P <0.05) more frequently increased than all other markers tested (Table 3).

Diagnostic Performance of GPBB

The ROC curves of GPBB, CKMB mass, CK, and cTnT for the discrimination between patients without and with acute ischemic coronary syndromes (unstable angina at rest with transient ST-T alterations and AMI) are shown in Fig. 7. The GPBB ROC curve confirmed our URL in nontraumatic chest pain patients. The point on the plot with a 45° tangent and the greatest deviation from the diagonal line was 7 µg/L. The position of two (6 and 9 µg/L) other discrimination limits is also shown on the plot. With the use of 6 instead of 7 µg/L the sensitivity increases but, not surprisingly, specificity decreases as well. The discriminatory power of GPBB to detect acute ischemic coronary syndromes in patients at admission to the emergency department is superior to all other markers tested (Fig. 7, Table 4). The area under the GPBB ROC curve, 0.91 (95% confidence interval, 0.85–0.97), was
Table 2. Characteristics of time courses of markers in blood samples of patients with AMI who were admitted to the coronary care unit within 4 h after onset of chest pain.

<table>
<thead>
<tr>
<th></th>
<th>GPBB mass</th>
<th>Myoglobin mass</th>
<th>cTnT mass</th>
<th>CKMB mass</th>
<th>CK activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>First appearance of increased plasma values, h*</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
<td>4.5</td>
<td>6.0</td>
</tr>
<tr>
<td>Time to peak, h*</td>
<td>(2.0 - 4.0)</td>
<td>(3.0 - 5.0)</td>
<td>(3.0 - 6.0)</td>
<td>(3.0 - 7.3)</td>
<td>(4.3 - 8.0)</td>
</tr>
<tr>
<td>Return to normal, h*</td>
<td>8.0</td>
<td>5.0</td>
<td>12.5</td>
<td>9.5</td>
<td>11.5</td>
</tr>
<tr>
<td>Magnitude of increase at first appearanceb</td>
<td>(5.0 - 11.8)</td>
<td>(4.3 - 7.8)</td>
<td>(11.3 - 13.8)</td>
<td>(8.3 - 13.8)</td>
<td>(9.0 - 12.8)</td>
</tr>
<tr>
<td>Magnitude of increase at time to peakb</td>
<td>(31.0 - 45.0)</td>
<td>(9.0 - 19.8)</td>
<td>(60.0 - 75.0)</td>
<td>(40.5 - 60.0)</td>
<td>(38.0 - 57.5)</td>
</tr>
</tbody>
</table>

* Calculated from the onset of chest pain.

b Calculated by dividing peak values (µg/L or URL) by upper reference limits.

All patients received intravenous thrombolytic treatment within 4 h after the onset of chest pain.

significantly (P ≤0.012) greater than the areas under all other ROC curves (see Fig. 7), i.e., 0.81 (0.73-0.89) for CKMB mass, 0.73 (0.65-0.83) for cTnT, and 0.69 (0.59-0.79) for CK. All other areas did not differ significantly, except for CKMB mass vs CK (P = 0.011). Various indicators describing the performance of a biochemical marker to diagnose acute ischemic coronary syndromes are listed in Table 4. The URLs of markers were used as cutoff values for the calculation of the values listed in Table 4. The distributions of GPBB mass, CKMB mass, CK, and cTnT concentrations investigated in the blood of 107 patients on admission to the emergency room (Table 5) explain the superiority of GPBB over the other tested markers for the diagnosis of acute coronary syndromes. This superiority is based on, first, GPBB’s higher early sensitivity for AMI (Tables 3 and 5), and second, its early increase in patients with unstable angina and transient ST-T alterations (Table 5). In the latter patients, GPBB was the only marker that was not only significantly higher (P = 0.0001) than in patients without ST-T changes, but was also increased above its URL in the majority of these patients even on admission to the emergency department (Table 5). By contrast, CK activities, CKMB mass, and cTnT concentrations were still within the reference interval in most of these patients. The potential usefulness of GPBB for diagnosing and monitoring unstable angina is also demonstrated by Fig. 8. This figure shows GPBB, CKMB mass, CK, myoglobin, and cTnT time courses of a typical patient with unstable angina at rest and ST-T alterations in whom we could obtain serial samples during his stay at the coronary care unit.

Table 3. Early sensitivities of biochemical markers in 22 AMI patients before thrombolytic therapy.

<table>
<thead>
<tr>
<th>Hours after onset of symptoms</th>
<th>GPBB mass</th>
<th>Myoglobin mass</th>
<th>CK activity</th>
<th>CKMB mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤4</td>
<td>0.77</td>
<td>0.36*</td>
<td>0.18*</td>
<td>0.41*</td>
</tr>
<tr>
<td></td>
<td>(0.55 - 0.92)</td>
<td>(0.17 - 0.59)</td>
<td>(0.05 - 0.40)</td>
<td>(0.21 - 0.64)</td>
</tr>
<tr>
<td>&lt;2</td>
<td>0.70</td>
<td>0.1*</td>
<td>0.1*</td>
<td>0.1*</td>
</tr>
<tr>
<td></td>
<td>(0.35 - 0.93)</td>
<td>(0.0 - 0.45)</td>
<td>(0.0 - 0.45)</td>
<td>(0.0 - 0.45)</td>
</tr>
<tr>
<td>≥2 - ≤4</td>
<td>0.83</td>
<td>0.58</td>
<td>0.25*</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>(0.52 - 0.98)</td>
<td>(0.28 - 0.85)</td>
<td>(0.05 - 0.57)</td>
<td>(0.35 - 0.90)</td>
</tr>
</tbody>
</table>

Two-sided P values were analyzed by using 2 × 2 contingency table and Fisher’s exact test, respectively. Values are significantly different from GPBB at * P < 0.05 and b P < 0.01.

Fig. 7. ROC curves of GPBB, cTnT, CKMB mass, and CK activity for identification of acute ischemic coronary syndromes in 107 emergency room patients with chest pain.

Diseases include Q-wave (n = 24) and non-Q-wave (n = 21) infarction, unstable angina pectoris with (n = 17) and without (n = 32) transient ST-T alteration, and chest pain without CAD (n = 13). Blood samples were taken on admission of patients. Patient characteristics and calculation of ROC curves and areas under the ROC curves are given in Subjects and Methods. The diagonal line represents a worthless test (sensitivity = 1 - specificity); the greater the deviation from this line, the better the discriminatory potency of the marker. For GPBB, the localizations of the URL (7 µg/L) and two other discrimination limits are indicated. The point with a 45° tangent on the GPBB plot is 7 µg/L. Prevalence of acute coronary syndromes (CAD): 0.56.

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Table 4. Diagnostic performances* of markers in diagnosis of acute ischemic coronary syndromes in nontraumatic chest pain patients on admission to emergency room.

<table>
<thead>
<tr>
<th></th>
<th>GPBB mass</th>
<th>cTnT mass</th>
<th>CKMB mass</th>
<th>CK activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (95% CI)</td>
<td>0.81</td>
<td>0.36</td>
<td>0.48</td>
<td>0.39</td>
</tr>
<tr>
<td>Specificity (95% CI)</td>
<td>0.93</td>
<td>0.91</td>
<td>0.96</td>
<td>0.84</td>
</tr>
<tr>
<td>Efficiency (95% CI)</td>
<td>0.86</td>
<td>0.59</td>
<td>0.67</td>
<td>0.58</td>
</tr>
<tr>
<td>Positive predictive value (95% CI)</td>
<td>0.94</td>
<td>0.85</td>
<td>0.94</td>
<td>0.77</td>
</tr>
<tr>
<td>Negative predictive value (95% CI)</td>
<td>0.78</td>
<td>0.51</td>
<td>0.67</td>
<td>0.50</td>
</tr>
<tr>
<td>Positive likelihood ratio (95% CI)</td>
<td>11.6</td>
<td>4.0</td>
<td>12.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Negative likelihood ratio (95% CI)</td>
<td>0.20</td>
<td>0.70</td>
<td>0.54</td>
<td>0.73</td>
</tr>
</tbody>
</table>

* Based on the URLs of the biochemical markers. Prevalence of acute ischemic coronary syndromes: 0.58.

Discussion

Here we mainly describe the development and evaluation of a new assay for measuring human GPBB. We also summarize our first results of the clinical evaluation of the marker and review the pathophysiological background of GPBB. Clinical key features of the marker (early sensitivity for AMI, early release in patients with severe unstable angina pectoris) have already been published (9, 43). For the most part the subjects of previous investigations (9, 42, 43) were included in the larger patient populations of this study.

IEMA of GPBB

The mAb combination chosen allowed the development of a sensitive and specific IEMA for the detection of human GPBB. The analytical sensitivity, cross-reactivities with other isoenzymes of GP, linearity, recovery, and precision of the assay are satisfactory. With this assay GPBB basal concentrations in healthy individuals and patients without acute ischemic heart diseases were low, which is a prerequisite for a high diagnostic sensitivity. We calculated a URL of 7 µg/L for measurement of GPBB in EDTA-plasma of 116 healthy subjects. This limit could be confirmed in other reference populations such as patients with chronic stable angina pectoris and nontraumatic chest pain patients. The latter may be the ideal subjects to meet the demand for similarity of preanalytical conditions between patients and reference subjects, because they were hospitalized with chest pain but later proved not to have had an acute ischemic coronary syndrome.

Table 5. Comparison of markers in nontraumatic chest pain patients on admission to emergency room (n = 107).

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>Delay, h*</th>
<th>GPBB mass, µg/L</th>
<th>cTnT, µg/L</th>
<th>CKMB mass, µg/L</th>
<th>CK activity, U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q-wave AMI</td>
<td>24</td>
<td>2.0</td>
<td>28.5</td>
<td>0.28</td>
<td>8.4</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.0 - 3.9)</td>
<td>(16.8 - 42.7)</td>
<td>(0.19 - 0.56)</td>
<td>(3.9 - 19.0)</td>
<td>(37 - 102)</td>
</tr>
<tr>
<td>Non-Q-wave AMI</td>
<td>21</td>
<td>5.0</td>
<td>12.9</td>
<td>0.58</td>
<td>21.4</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.6 - 8.8)</td>
<td>(4.8 - 19.0)</td>
<td>(0.29 - 0.70)</td>
<td>(6.6 - 42.5)</td>
<td>(39 - 147)</td>
</tr>
<tr>
<td>UA with ST-T changes</td>
<td>17</td>
<td>3.5</td>
<td>12.2</td>
<td>0.22</td>
<td>3.9</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.8 - 6.0)</td>
<td>(8.5 - 19.3)</td>
<td>(0.19 - 0.35)</td>
<td>(2.7 - 5.7)</td>
<td>(28 - 68)</td>
</tr>
<tr>
<td>UA without ST-T changes</td>
<td>32</td>
<td>2.5</td>
<td>4.0</td>
<td>0.17</td>
<td>2.4</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.5 - 5.1)</td>
<td>(2.9 - 4.5)</td>
<td>(0.12 - 0.23)</td>
<td>(1.9 - 3.1)</td>
<td>(19 - 50)</td>
</tr>
<tr>
<td>Chest pain, no CAD</td>
<td>13</td>
<td>2.5</td>
<td>3.5</td>
<td>0.23</td>
<td>3.1</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.8 - 6.0)</td>
<td>(3.2 - 6.9)</td>
<td>(0.13 - 0.29)</td>
<td>(1.6 - 5.5)</td>
<td>(18 - 61)</td>
</tr>
</tbody>
</table>

* The time from onset of symptoms to admission. UA, unstable angina.
GPBB as Marker of Ischemic Myocardial Injury

GP has three isoenzymes. The presented GPBB IEMAs do not show substantial cross-reactivity with other isoenzymes such as GPM and GPLL (<1% each). Brain and myocardium are the only known tissues with considerable GPBB content (17, 18) and, therefore, increases in GPBB should be highly specific for ischemic myocardial injury when damage to the brain and consequent disturbance of the blood–brain barrier can be excluded. We found a high specificity of GPBB that was in the range of that of CKMB or cTnT in emergency department patients presenting with chest pain. This patient population was preselected, and, therefore, future studies on the diagnostic specificity of GPBB will also have to address the issue in an unselected cohort of patients that includes severely traumatized patients with and without head injuries and patients with liver damage or renal failure. However, the most striking and promising feature of GPBB is its high sensitivity for AMI during the very early hours after the onset of chest pain. For the first time we compared the early sensitivities of GPBB, CKMB mass, myoglobin, and cTnT. The same patients were tested for all biochemical markers, which is a prerequisite for a good, reasonable comparison of concentration time courses of different markers. Our study population was not uniform (e.g., a mixed cohort of Q-wave and non-Q-wave AMI, some scatter in the delay from the onset of symptoms to admission, differences in the reperfusion status of the infarct-related coronary artery). Because we tested and compared all markers in the same AMI cohort, all these possible biases affect all biochemical markers tested in a similar manner; therefore, they may limit but do not qualitatively upset our results, which allow several important conclusions. This is highlighted by the fact that results and conclusions derived from samples drawn before thrombolytic therapy were identical. Our study confirms that the sensitivity of conventional markers, including myoglobin, CKMB mass, and cTnT, is not sufficient during the first 2 h after the onset of chest pain (24, 25, 27, 44, 45). However, there were distinct differences in sensitivities of GPBB in comparison with myoglobin, CKMB mass, CK, and cTnT within the first 2 h after AMI onset, and in our patients GPBB was the most sensitive marker during the first 4 h after AMI onset. In contrast to all other markers tested, GPBB started to increase during the first 2 h after the onset of chest pain in the majority of AMI patients. Therefore, it may be a very important marker for the early diagnosis of AMI. However, this high early sensitivity requires the development of a rapid assay suitable for stat determination in the routine emergency laboratory. In addition, larger series of more uniform AMI patients must be investigated to confirm the diagnostic validity of GPBB before GPBB determination can be introduced as a part of the routine evaluation of patients with suspected AMI who are admitted within 3 h after the onset of chest pain.

Similar to soluble markers such as myoglobin and CKMB (5), we could demonstrate that GPBB time courses in AMI patients are markedly influenced by whether early reperfusion of the infarct-related coronary artery occurs. The well-established “wash-out” phenomenon after successful thrombolysis leads to a more rapid increase in GPBB, with earlier and higher peak values. Therefore, GPBB may be useful, along with other soluble myocardial proteins (5), for assessing the effectiveness of thrombolytic therapy noninvasively. However, decision limits to detect successful and, clinically more important, failed reperfusion remain to be established in an acutely performed CAG controlled study. To determine the exact timing, character, and degree of reperfusion requires serial acute CAGs, which could not be done in this study. Our study reports preliminary data on the reperfusion-dependent release of GPBB after AMI.

GPBB application is not restricted to conventional AMI. As underlined by the ROC curve and ROC area calculations of GPBB and comparison with those of the other markers studied, GPBB showed the best diagnostic performance of all markers tested to detect acute ischemic coronary syndromes (AMI, or severe unstable angina at rest with transient ST-T alterations) on admission. Previously, an increase in CKMB mass, myoglobin, and cTnT concentrations has been described in serially drawn blood samples of a subgroup of patients with unstable angina (46–49). Confirming earlier results (43), we found an early release of GPBB in the blood of patients with unstable angina and transient ST-T alterations. In these patients GPBB was the only marker that was increased above its URL on admission to the emergency department. In the present study, the patients without ischemic heart disease and the healthy individuals represent the background GPBB plasma concentration, and the AMI patients represent the full extent of GPBB release.
when there is irreversible cell damage. These are ideal reference groups for the study of GPBB concentrations in patients with angina pectoris. GPBB plasma concentrations in patients with chronic stable angina or unstable angina without ST-T changes at rest resembled those of healthy individuals or patients without angina. However, in patients with unstable angina at rest and transient ST-T alterations, we found an early release of GPBB. In addition, fluctuitions of GPBB found in a patient with unstable angina in whom we could obtain serial samples correlated with evidence of repetitive ischemic episodes. GPBB may reflect the degree of myocardial damage. Whether GPBB release is due to minimal necrosis of myocardial tissue or severe reversible ischemic injury cannot be determined from the present study. In unstable angina a dynamic stenosis can cause unpredictable episodes of myocardial ischemia. Thus, the challenge is not always simply to rule AMI in or out, but rather to distinguish early the patients with acutely unstable coronary lesions from those with either stable coronary lesions or none. The results of the present study suggest that for this purpose GPBB may be superior to conventional markers, including myoglobin, CKMB mass, and cTnT. GPBB IEMA is a promising diagnostic tool, because it may help to determine the frequency and severity of myocardial ischemic events in the different coronary syndromes.

Myocardial Oxygen Deficiency and GPBB Release

The early release and the high diagnostic early sensitivity of GPBB for severe myocardial ischemia and AMI raise questions on the mechanisms of GPBB release from ischemic myocardium, particularly considering the higher molecular mass of GPBB (188 kDa as dimer) compared with myoglobin (17.8 kDa), cTnT (37 kDa), and CK (86 kDa as dimer). An essential part of a possible explanation may be its key role in the energy metabolism of ischemic myocardium. A substantial amount of phosphorylase activity in the heart muscle is normally associated with microsomal membranes together with glycogen particles (20, 21, 50–52), an SR–glycogenolysis complex being assumed (21, 22) to represent a functionally coupled association. With isolated glycogen particles, a burst in glycogenolysis could be initiated by either calcium ions or cAMP (22). During acute ischemia a sympathetic activation of the myocardium is followed by a transient rise in cardiac cAMP concentrations and by activation of GP via conversion of the nonphosphorylated b form into phosphorylase a (53–55). Concomitantly the rate of glycogenolysis is accelerated (6, 55). Kinetic properties of GPBB also allow a glycogen breakdown catalyzed by the b form, as this isoenzyme is characterized by low values of $K_m$ for the substrate orthophosphate as well as of $K_{0.5}$ for the activator AMP, in comparison with those known for human GPMM (16). An ischemia-induced rise in the concentrations of intracellular orthophosphate and AMP may therefore induce a second, long-lasting acceleration of glycogenolysis under these conditions. Indeed, cardiac glycogen breakdown was found to be continued during postischemic reperfusion when the a form of GP declined to preischemic control concentrations but the orthophosphate concentration was still high (55).

The first indication that GP can be released from damaged muscle tissue into blood was obtained from rabbit after removal of a tourniquet that had compressed the hind leg for several hours (56). An efflux of GP has been also observed in isolated perfused rabbit heart after interruption of perfusion (7, 23). In conscious dogs a rapid release of GPBB and CKMB was measured in the cardiac lymph after a transient ligation of the coronary artery ≤10 min (6). In these experiments as well as in patients with AMI, the released GPBB was exclusively found in the b form (6, 57). Thus the activity of GPBB (form b) may catalyze the degradation of glycogen in the SR–glycogenolysis complex in the ischemic area of the myocardium (54, 58). When glycogen is broken down and disappears, GPBB becomes free to move from the peri-SR compartment directly into the extracellular fluid, if cell membrane permeability is simultaneously increased, which is usually the case in ischemia. A gradient in GPBB concentration, which immediately forms in this particular compartment of the SR–glycogenolytic complex (Fig. 9), may be the reason for the

![Fig. 9. GPBB release from the cardiomyocyte in ischemia.](image-url)
high efflux rate of GBPB, which, contrary to other cytosolic constituents, may be realized, at least partly, via T-tubuli. The fact that the sensitive glycogen degration regulated by Ca²⁺, metabolic intermediates, and catecholamines seems to be a crucial prerequisite for the efflux of GBPB thus underlines the specific sensitivity of this enzyme marker to indicate transient imbalances in heart energy metabolism, as is the case during angina pectoris attacks and (or) in the infarcting myocardium.

Whether an increase of GBPB may occur in response to therapeutic circumstances in which cardiac work is increased and glycogen might be mobilized, such as after administration of catecholamines and glucagon, is very unlikely so long as concomitant myocardial injury is not processed. In experimental data, even high doses of epinephrine did not cause a detectable GP release from cardiac myocytes without cell membrane damage (23).

In summary, we report a sensitive and specific IEMA for the determination of human GBPB. GBPB was the most sensitive biochemical marker of all tested (CK, CKMB mass, myoglobin, cTnT) in AMI patients during the first 4 h after the onset of chest pain. Application of GBPB is not restricted to conventional AMI. GBPB was markedly increased in the majority of patients with unstable angina and reversible ST-T changes even on admission. It may reflect, in time and degree, the range of ischemic damage in myocardial tissue. GBPB IEMA is a promising means of extending our knowledge of the severity of myocardial ischemic events in the various coronary syndromes. The high early sensitivity of GBPB for ischemic myocardial injury is probably explained by its function as a key enzyme of glycogenolysis.

We are indebted to C.E. Metz (Department of Radiology, University of Chicago, Chicago, IL) for providing the software needed for ROC analysis and comparison. We dedicate this paper to Herbert Keller (St. Gallen/Zürich, Switzerland) on the occasion of his 70th birthday.

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