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Glycogen phosphorylase isoenzyme BB in diagnosis of myocardial ischaemic injury and infarction

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Abstract

This review deals with glycogen phosphorylase (GP) and its isoenzyme BB in the diagnosis of ischaemic myocardial injury. Early identification and confirmation of acute myocardial infarction is essential for correct patient care and disposition decision in the emergency department. In this respect, glycogen phosphorylase isoenzyme BB (GPBB) based on its metabolic function is an enzyme for early laboratory detection of ischaemia. In the aerobic heart muscle GPBB together with glycogen is tightly associated with the vesicles of the sarcoplasmic reticulum. Release of GPBB, the main isoform in the human myocardium, essentially depends on the degradation of glycogen, which is catalyzed by GP. Ischaemia is known to favour the conversion of bound GP in the *b* form into GP *a*, thereby accelerating glycogen breakdown, which is the ultimate prerequisite for getting GP into a soluble form being able to move freely in the cytosol. The efflux of GPBB into the extracellular fluid follows if ischaemia-induced structural alterations in the cell membrane become manifest. The clinical application of GPBB as a marker of ischaemic myocardial injury is a very promising tool for extending our knowledge of the severity of myocardial ischaemic events in the various coronary syndromes. The rational roots of this development were originated from Albert Wollenberger's research work on the biochemistry of cardiac ischaemia and the transient acceleration of glycogenolysis mainly brought about by GP activation. (Mol Cell Biochem **160/161**: 289–295, 1996)

Key words: myocardial infarction, glycogen phosphorylase, isoenzyme BB, glycogenolysis, enzyme release, glycogen-glycogenolysis-complex, cardiac ischaemia.

Introduction

During recent years biochemical markers for the detection of acute myocardial injury gained particular interest for the early diagnosis of evolving acute myocardial infarction (AMI), the monitoring of thrombolytic treatment, and for risk stratification in patients with unstable angina. Creatine kinase (CK) MB mass, CK isoforms, myoglobin, cardiac troponin I and troponin T have a comparable early sensitivity for AMI, and all are markedly more sensitive than CK and CKMB activity [1]. However, in the final analysis myoglobin, CKMB mass, CK isoforms, and troponins are not sufficiently sensitive within the first 3–4 hours after the onset of AMI, and the diagnostic performance of the electrocardiography (ECG) is clearly superior to that of biochemical markers during this time interval [1, 2]. However, up to 50% of chest pain patients may have non-diagnostic ECGs at hospital admission [3]. Laboratory parameters are cheaper, easier to perform and to interpret for the non-specialist than other alternative diagnostic methods (e.g. echocardiography, myocardial scintigraphy). Consequently the search is still going on for more rapidly detectable and more sensitive markers which should start to be released in the phase of reversible ischaemic myocardial damage. In this respect, glycogen phosphorylase isoenzyme BB (GPBB) based on its metabolic function and on first clinical results is a very promising enzyme for the early laboratory detection of ischaemic myocardial injury.

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Biochemistry of glycogen phosphorylase

Glycogen phosphorylase (GP) is one of the best studied enzymes in biochemistry. It is a glycolytic enzyme which plays an essential role in the regulation of carbohydrate metabolism by mobilization of glycogen [4]. It catalyses the first step in glycogenolysis in which glycogen is converted to glucose-l-phosphate, utilising orthophosphate. The physiological role of muscle phosphorylase is to provide the fuel for the energy supply required for muscle contraction. Its activity is allosterically regulated by the binding of AMP and phosphorylation. Phosphorylase kinase converts GP *b* into its more active form GP *a*. Phosphorylase exists in the cardiomyocyte in association with glycogen and the sarcoplasmatic reticulum and forms a macromolecular complex (sarcoplasmatic reticulum glycogenolysis complex) [5, 6]. The degree of association of GP with this complex depends essentially on the metabolic state of the myocardium. With the onset of tissue hypoxia, when glycogen is broken down and disappears, glycogen phosphorylase is converted from a particulate into a soluble form, and the enzyme becomes free to move around in the cytoplasma [5–7].

GP exists as a dimer under normal physiological conditions. The dimer is composed of 2 identical subunits. At least 3 GP isoenzymes are found in human tissues that are named after the tissue in which they are preferentially expressed, GPLL (liver), GPMM (muscle), and GPBB (brain) [4]. The three isoenzymes can be distinguished by functional and immunological properties. They are encoded by three distinct genes. The genes of the 3 human GP isoenzymes have been cloned and sequenced [8]. The proteins predicted by the cDNA sequences consist of chains of 846 (LL), 842 (MM) and 862 (BB) amino acid residues. Amino acids 1–830 match and differences are mainly found at the C-terminus, which is the catalytic domain of the protein. In pairwise sequence comparison the brain type protein is 80% identical with the liver type and 83% identical with the muscle type. GPBB has 21 and 16 additional amino acid residues on its C-terminal portion that are not present on the MM and LL isoenzymes, respectively.

Adult human skeletal muscle contains only one isoenzyme, *GPMM. GPLL* is the predominant isoenzyme in human liver and all other human tissues except heart, skeletal muscle, and brain. The *isoenzyme BB* is the predominant isoenzyme of human brain. Its molecular weight as a monomer is approximately 94 kD. In the human heart the isoenzymes BB and MM are found, but GPBB is still the predominant isoenzyme in myocardium. By far the highest concentrations of GPBB were found in human brain and heart. The tissue concentrations of GPBB in heart and brain are comparable [9]. Although immunoblot, electrophoresis, and northern blot data are partly conflicting [4, 8–11], there is evidence that GPBB isoenzyme might not be restricted to brain and heart in humans. Much lower GPBB concentrations have been reported, for example, in leukocytes, spleen, kidney, bladder, testis, digestive tract and aorta. However, in all these tissues the isoenzyme LL is by far the predominant GP isoenzyme.

Pathophysiology: Shift to anaerobic energy production in the acutely ischaemic myocardium, activation and release of GP

The energy metabolism of the heart, in particular the biochemistry of cardiac ischaemia as well as the biochemical mechanisms of the neuronal and hormonal regulation of the heart became a main profile of Albert Wollenberger's research work and his group during the sixties [12–17]. Investigating the energy production in the acutely ischaemic myocardium Wollenberger *et al.* [12] demonstrated that in ischaemia the sources of utilizable chemical energy (ATP, phosphocreatine) are rapidly exhausted. They clarified the mechanism and demonstrated the rapidity of cardiac glycogenolysis when the blood circulation was suddenly arrested [13–15]. Of course, the activation of conversion of glycogen to glucose phosphate cannot be faster than the velocity of the first reaction step in this process which is catalyzed by GP. Using the freeze-clamp technique [16] a transient rise in cardiac cAMP levels followed by an activation of GP due to conversion of the nonphosphorylated *b* form into phosphorylase *a* by phosphorylase kinase was for the first time demonstrated during acute ischaemia (Fig. 1) [17]. As β-adrenoceptor blockade diminished the GP conversion a sympathetic activation of adenylyl cyclase by neurotransmitters was postulated at that time [14, 17]. Concomitantly the rate of glycogenolysis was found to be accelerated [13–15]. Furthermore there is evidence that the kinetic properties of GPBB allow a glycogen breakdown catalyzed by the *b* form. Compared with GPMM 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 [10]. An ischaemia-induced rise in the levels of intracellular orthophosphate and AMP in myocardium may therefore induce a second, long lasting acceleration of glycogenolysis under these conditions [15, 18]. Indeed cardiac glycogen breakdown was found to continue during post-ischaemic reperfusion when the *a* form of GP declined to pre-ischaemic control levels when the orthophosphate level was still high [19]. Using isolated glycogen particles form canine heart muscle a burst in glycogenolysis could be initiated by either cAMP and calcium ions which was accompanied by a breakdown of glycogen [7]. In experimental studies as well as in patients with acute myocardial infarction the released GPBB was exclusively found in the *b* form [20, 21]. Thus it is suggested that the activity of GPBB (form *b*) catalyses the prolonged degradation of glycogen in the sarcoplasmatic reticulum-glycogenolysis complex in the

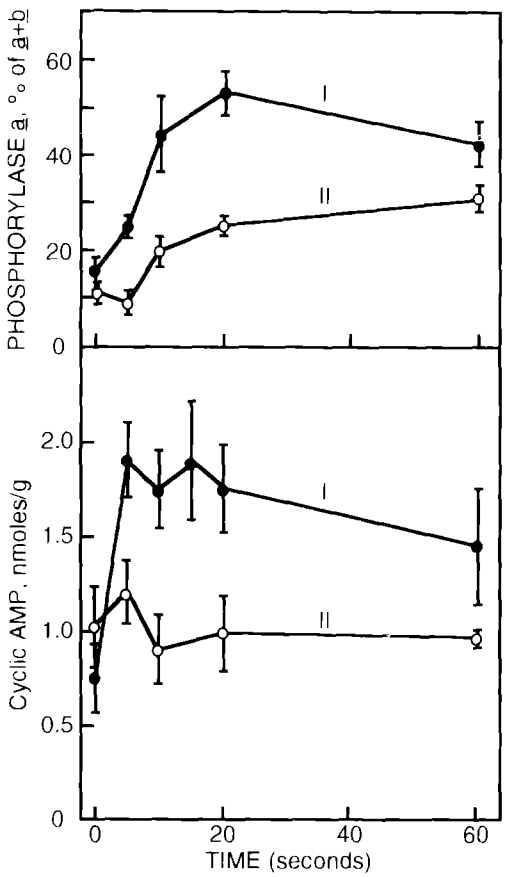

Fig. 1. Cyclic AMP and phosphorylase a levels in the left ventricle of the dog before and after the onset of myocardial ischaemia. I: Dogs not treated with a β-blocker; II: dogs treated with pronethalol. Phosphorylase (a + b) content averaged 14.3 μmole phosphate/g × min in both group I and group II. Adapted from Wollenberger *et al.* [17]

ischaemic area of the myocardium [15, 18].

In conscious dogs a rapid release of GPBB was measured in the cardiac lymph after a transient ligation of a coronary artery for not longer than 10 min, which did not lead to histological signs of myocardial necrosis [20]. An efflux of GP from the myocardium after hypoxia or substrate depletion has been observed earlier in the isolated perfused rat and rabbit heart (Fig. 2) [22, 23]. The GP release in these experiments correlated with the remaining myocardial glycogen content [23]. In this Langendorff model of the isolated perfused heart the addition of imipramine under aerobic conditions to a cardioplegic perfusion solution only caused a release of CK, but not of GP (Fig. 3) [23]. The myocardial glycogen content remained uneffected as well. Imipramine causes in a certain concentration range a selective increase in the plasma membrane permeability without myocardial hypoxia. On the other hand, the stimulation of glycogenolysis by high doses of adrenaline did not cause a decrease in myocardial GP activity, although the glycogen content of the tissue was greatly diminished by the added adrenaline (Table 1) [22]. These experimental results allow one to conclude that the release of GPBB requires both a burst in glycogenolysis and a concomitantly increased plasma membrane permeability as known for injured cardiomyocytes by ischaemia [24].

Given its molecular mass (94 kD as a monomer) the early release of GPBB raises questions about the mechanisms of its release from ischaemic myocardium. An essential part of an explanation may be its key role in the energy metabolism of ischaemic myocardium. 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 ischaemia. A high GPBB concentration gradient, which immediately is formed in the compartment of the sarcoplasmatic reticulum glycogenolytic complex, may be the reason for the high efflux rate of this enzyme. In contrast to other cytosolic proteins, this gradient may at least partly be also realized via *T-tubuli* and may contribute to the efflux of GPBB (Fig. 4).

In summary, the ischaemia-sensitive glycogen degradation, which is regulated by Ca^{2+}, metabolic intermediates and catecholamines, seems to be a crucial prerequisite for the efflux of GPBB. This outlines the specific sensitivity of this enzyme marker to indicate transient imbalances in heart energy metabolism as it is the case during angina pectoris attacks and/or in the infarcting myocardium. Therefore this enzyme is a promising analyte for the detection of ischaemic myocardial injury.

First Clinical Results

Acute myocardial infarction

There have been distinct differences in sensitivities of GPBB in comparison with myoglobin, CKMB mass, CK, and cardiac troponin T within the first 2–3 h after AMI onset [25, 26], and in our patients GPBB was the most sensitive parameter during the first 4 h after AMI onset (Table 2). In the majority of AMI patients GPBB increased between 1–4 h after the onset of chest pain. Therefore, GPBB may be a very important marker for the early diagnosis of AMI. GPBB usually peaks before CK, CKMB or troponin T and returns within the reference interval within 1–2 days after AMI onset (Fig. 5). Basal concentration of GPBB in blood plasma of normal healthy was found to be independent of sex and age; the empirical data distribution of basal GPBB was gaussian [27].

As with soluble markers, such as myoglobin and CKMB, we could demonstrate that time course of GPBB in AMI patients is markedly influenced by whether or not early reperfusion of the infarct-related coronary artery occurs [26]. The well established so called 'wash out' phenomenon after successful thrombolysis leads to a more rapid increase in GPBB

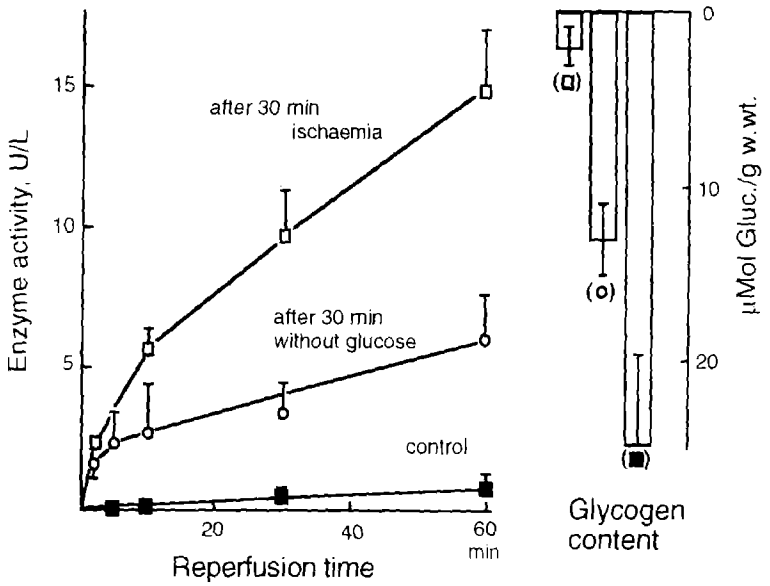

Fig. 2. Release of glycogen phosphorylase and glycogen content of rabbit hearts perfused in the Langendorff mode Enzyme efflux was assayed during reperfusion after global ischaemia (□), after substrate depletion (○), and under aerobic control perfusion conditions (■). Finally the perfused hearts were freeze-clamped and glycogen content was determined. Values are means plus 1 S.E.M. Gluc. = glucosyl residue; W. Wt. = wet weight. Adapted from Krause *et al.* [23].

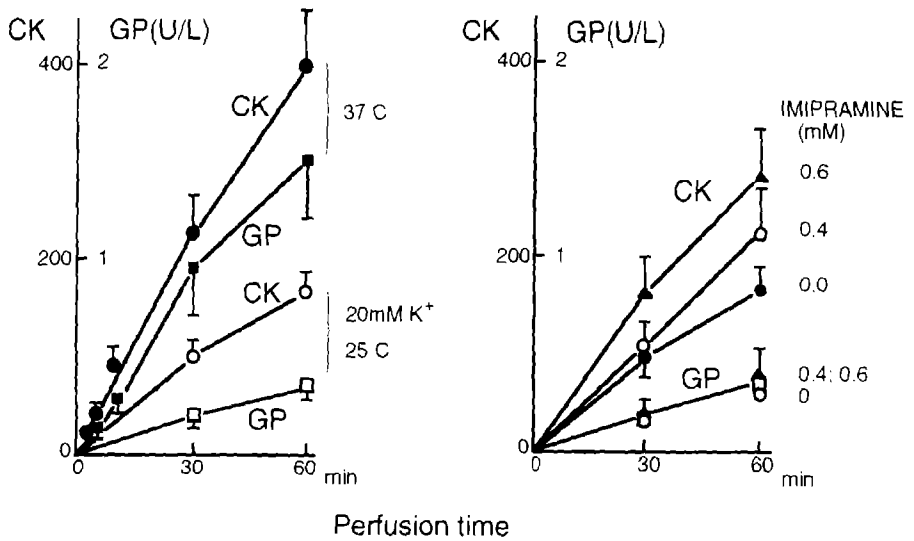

Fig. 3. Release of glycogen phosphorylase (GP) and creatine kinase (CK) from normothermic beating and hypothermic, K^+-arrested, perfused rabbit hearts. The effect of 0.4 and 0.6 mM imipramine was only studied with non-beating hearts at 24°C. Adapted from Krause *et al.* [23].

and results in earlier and higher peak values (Fig. 6). Therefore, GPBB may be useful, alongside with other soluble myocardial proteins, to assess the effectiveness of thrombolytic therapy non-invasively. However, decision limits to detect successful, and what is clinically more important, failed reperfusion, remain to be established in a study controlled with acutely performed coronary angiography.

Unstable angina pectoris

The application of GPBB is not restricted to conventional myocardial infarction. An early release of GPBB was demonstrated in patients with Braunwald class III unstable angina who showed ST-T alterations at rest. Only GPBB was increased above the upper reference limit in the majority of these patients at hospital admission (Fig. 7) [28]. Whether the early GPBB release in these patients was due to minimal necrosis of myocardial tissue or severe reversible ischaemic

Table 1. Activity of glycogen phosphorylase (GP) and glycogen concentration in perfused rat hearts after addition of adrenaline

Treatment	GP a+b (μmol orthophosphate/ min × mg protein)	Glycogen (μmol glucose/ g wet weight)
Controls (n=5)	0.41 ± 0.05	13.4 ± 2.4
Adrenaline (n=5)	0.50 ± 0.09	3.9 ± 0.8

Adapted from Schulze *et al.* [22].

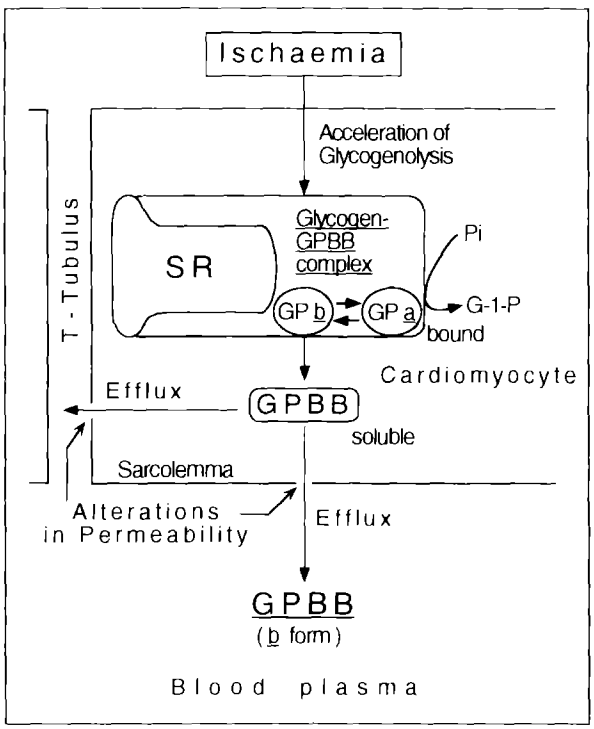

Fig. 4. Scheme of GPBB release from myocardium in ischaemia. Glycogen phosphorylase (GP) together with glycogen is tightly associated with the vesicles of sarcoplasmic reticulum (SR) under normal conditions. A release of GPBB, the main isoform in the myocardium, essentially depends on the degradation of glycogen, which is catalyzed by GP *a* (the phosphorylated, active form of the isoenzyme) and by GP *a* (nonphosphorylated, AMP-dependent form). Ischemia is known to favor the conversion of bound GP *b* into GP *a* thereby accelerating glycogen breakdown, which seems to be the ultimate prerequisite for getting GP into a soluble form. An efflux of GPBB into the extracellular fluid may only follow if ischaemia-induced structural alterations in the cell membrane are manifested. Pi: inorganic phosphate; G-1-P: glucose1-phosphate. For more details see text. Adapted from Rabitzsch *et al.* [26].

injury is currently not known. As underlined by the receiver-operating characteristic (ROC) curve and ROC area calculations of GPBB and comparison with those of CK, CKMB mass, myoglobin, and troponin T [26], GPBB showed the best diagnostic performance of all markers tested to detect acute ischaemic coronary syndromes (AMI or severe unstable angina at rest with transient ST-T alterations) on admission (Fig. 8). GPBB plasma concentrations in patients with stable angina resembled those of healthy individuals or patients without angina [26].

Table 2. Early sensitivities of GPBB and other biochemical markers before start of thrombolytic treatment in patients with acute myocardial infarction who were admitted within 4 h after the onset of chest pain

	GPBB mass	CK	CKMB mass	Myoglobin	Troponin T
Sensitivity	0.77 (0.55–0.92)	0.20 (0.04–0.48)	0.47 (0.21–0.73)	0.47 (0.21–0.73)	0.40 (0. 16–0.68)

(95% confidence interval)

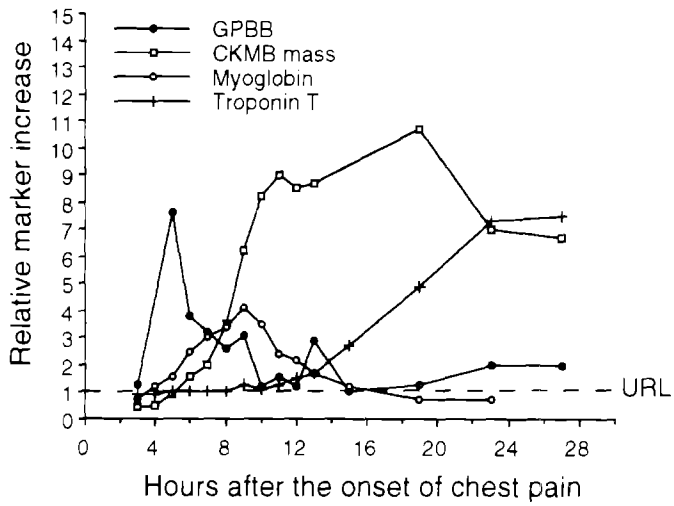

Fig. 5. Glycogen phosphorylase BB, CKMB mass, myoglobin, and cardiac troponin T time courses in a patient with a small non-Q wave myocardial infarction. Data are given as ×-fold increase of the upper reference limit (URL).

Coronary artery bypass grafting (CABG)

GPBB is also a sensitive marker for the detection of perioperative myocardial ischaemia and infarction in patients undergoing CABG [29]. In uncomplicated patients GPBB peaks within 4 h after aortic unclamping and returns to baseline values within 20 h. GPBB release correlates with aortic crossclamping time, which reflects the duration of myocardial hypoxia during cardioplegic cardiac arrest. GPBB time courses of patients with perioperative myocardial infarction (PMI) differ markedly in time to peak values (peaks occur later) and peak concentrations (> 50 μg/L) from uneventful patients. However, also patients with severe episodes of perioperative myocardial ischaemia that do not fulfil standard PMI criteria show markedly elevated GPBB concentrations compared with uncomplicated patients. In patients with emergency CABG GPBB, but not CKMB, correlated with clinical evidence of myocardial ischaemia [29]. In summary, GPBB is a very sensitive marker of perioperative ischaemic myocardial injury in CABG patients.

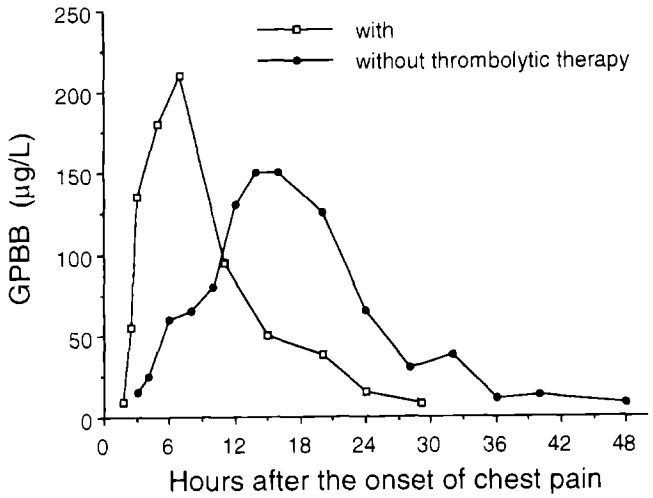

Fig. 6. Influence of early reperfusion of the infarct related coronary artery on glycogen phosphorylase BB time courses GPBB time course in a patient who was successfully reperfused by thrombolytic therapy and in a patient who did not receive fibrinolytic treatment because of contraindications. The GPBB upper reference limit is 7 µg/L.

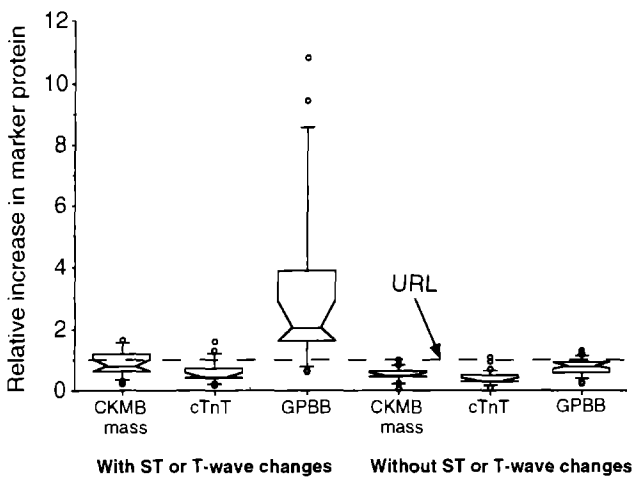

Fig. 7. CKMB mass. cardiac troponin T, and GPBB concentrations on admission to the emergency department in patients with unstable angina. Patients were grouped according to the presence of signs of myocardial ischaemia in the admission ECG recording. Marker concentrations are given as x-fold increase of the upper reference limit. The data are given as notched box plots. In the box plot the lines represent the 10th, 25th, 50th 75th, and 90th percentile, the notches the 95th confidence bands about the median, values above and below the 10th and 90th percentile are represented as data points. Abbreviations: creatine kinase (CK), cardiac troponin T (cTnT), glycogen phosphorylase (GP). Adapted from Mair *et al.* [28].

Diagnostic specificity

GPBB is not a heart-specific marker and its specificity is limited. However, increases in GPBB are specific for ischaemic myocardial injury when damage to the brain and consequent disturbance of the blood-brain barrier can be excluded. According to experimental studies and clinical observations increases in GPBB do not occur in response to therapeutic

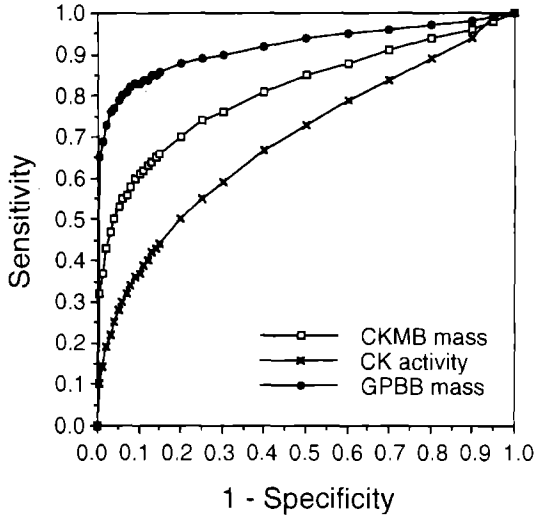

Fig. 8. Receiver operating characteristic (ROC), curves of GPBB. CKMB mass and CK activity for the identification of acute coronary syndromes in non-traumatic chest pain patients at hospital admission. An acute coronary syndrome was defined as either acute myocardial infarction or unstable angina with reversible ST-T alteration in the admission ECG recording. The larger the area under the ROC curve the better is the discriminating power of the parameter. The area under the GPBB ROC curve is significantly greater than those of the other markers. Adapted from Rabitzsch *et al.* [26].

circumstances in which cardiac work is increased and glycogen might be mobilized, such as after administration of catecholamines and glucagon, provided that a concomitant myocardial injury with cell membrane damage does not take place [22, 29]. The diagnostic specificity of GPBB for myocardial injury in non-traumatic chest pain patients was in the range of CKMB [26], which suggests sufficient specificity in clinical practice. Future studies on the diagnostic specificity of GPBB, however, will also have to address the issue in an unselected cohort of patients including severely traumatized patients with and without head injuries, patients with liver damage or renal failure. As long as the diagnostic specificity of GPBB for myocardial damage is not fully delineated, a positive GPBB result should be later confirmed by cardiac troponin I measurement.

Conclusions

The first hints that blood GP increases above its upper reference limit after the onset of AMI earlier than does CK were obtained approximately 2 decades ago, with total GP activity measured by a not very sensitive enzymatic assay [21]. The breakthrough occured later in our laboratory [25] with the development of a sensitive and specific immunoenzymometric assay for the measurement of the isoenzyme GPBB. This newly developed assay was used in all our clinical studies. The challenge still exists to develop a rapid assay that is suitable for bedside or 'stat' use in the routine laboratory. Of

course, the first clinical results will have to be confirmed in a larger number of patients but they allow several important conclusions, and there is no doubt that GPBB is a promising marker for the detection of ischaemic myocardial injury. This is probably explained by its function as a key enzyme of glycogenolysis. GPBB has so far been the most sensitive marker for the diagnosis of AMI within 4 h after the onset of chest pain. GPBB was the only marker which was increased in a considerable proportion of AMI patients within 2–3 hours after the onset of chest pain. The application of GPBB is not restricted to conventional AMI. GPBB also increased early in patients with unstable angina and reversible ST-T alterations in the resting ECG at hospital admission. Therefore GPBB could be useful for early risk stratification in these patients. GPBB has also been a sensitive marker for the detection of perioperative myocardial ischaemia and infarction in patients undergoing coronary artery bypass grafting. The diagnostic specificity of GPBB is sufficient for clinical practice, in non-traumatic chest pain patients it was in the range of that of CKMB. Thus, if the first clinical results on GPBB can be confirmed, the future scenario for the laboratory testing for myocardial injury could be the combination of cardiac troponin I or T and GPBB measurement, which combines cardiac specificity with high early sensitivity for ischaemic myocardial damage.

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