Biochemical mechanisms of hibernation and stunning in the human heart

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Abstract

Background: Myocardial hibernation and stunning are characterized by depressed cardiac function in the presence of reduced or normal coronary blood flow. The underlying biochemical mechanisms are widely unknown and only limited data are available in human hearts. Methods and Results: Left ventricular transmural myocardial biopsies were obtained from normal and dysfunctional segments of patients undergoing coronary bypass surgery. Segments were classified as hibernating (n=10) or stunned (n=9) using contrast ventriculography and echocardiography, single photon emission computed tomography (SPECT), and positron emission tomography (PET). In each patient, biopsies from normal myocardial segments were used as controls (n=19). Compared to control myocardium, levels of cAMP (3′-5′ cyclic adenosine monophosphate, in fmol/mg wet weight, means±S.E.M.) were higher in hibernating (673±76 versus 518±47, P<0.05) but unchanged in stunned myocardium (513±73 versus 466±97, P>0.05). Protein expression of phospholamban, sarcoendoplasmic Ca-ATPase 2a, calsequestrin, the inhibitory subunit of troponin, as well as the activation of p38 MAP kinase were not different when compared to controls. However, heat shock protein 72 (Hsp72) was increased 55% in stunned (2.89±0.58 versus 1.86±0.32, P<0.05) but not in hibernating myocardium (1.68±0.34 versus 1.67±0.29, P>0.05). Conclusions: The data from the present study suggest different pathophysiological mechanisms for myocardial hibernation and stunning. Alterations in the homeostasis of cAMP might be a compensatory mechanism in myocardial hibernation, whereas expression of Hsp72 appears to be cardioprotective in human myocardial stunning. Future studies should further elucidate these mechanisms and their potential impact on future therapeutic interventions.

Keywords: Calcium (cellular); Contractile function; Hibernation; Ischemia; Stunning

1. Introduction

Myocardial ischemia may lead to reversible ventricular dysfunction which may be present in two different conditions. Hibernation is defined as reversible dysfunction during severe chronic ischemia (reviewed in [1]), whereas stunning represents prolonged contractile depression after alleviation of severe ischemia (i.e. reperfusion after coronary occlusion) (reviewed in [2]).

In order to treat patients with myocardial hibernation and stunning properly, more insights into the underlying mechanisms are needed.
mechanisms in humans are warranted. Changes in the expression of Ca\(^{2+}\)-regulatory proteins have previously been suggested [3–5]. The expression of phospholamban, sarcopla-ndoplasmic Ca\(^{2+}\)-ATPase 2a (SERCA), calsequestrin (CSQ), and the inhibitory subunit of troponin (TnI) were investigated in a porcine model of short-term hibernation and stunning. Expression of these proteins was unaltered [6]. These findings were confirmed in a similar short-term model of hibernation in pigs [7]. However, others reported alterations in phospholamban, SERCA and CSQ after repetitive stunning in pigs [3]. This discrepancy can probably be explained by differences in the models used. Recently, a decrease in cAMP as well as an induction of the putative cardioprotective heat shock protein 72 (Hsp72) in canine stunned myocardium were found [8,9]. Furthermore, a transient activation of p38 MAP kinase during moderate low-flow ischemia in the porcine model of hibernation was noted [10]. From recent data, alterations of the β-adrenergic pathway might be involved in human hibernating myocardium [11]. Studies in transgenic animals clearly show that changes in the expression of Ca\(^{2+}\)-cycling proteins can decrease myocardial contractility: Increased protein levels of phospholamban and CSQ as well as decreased protein levels of SERCA resulted in impaired cardiac function, and TnI gene ablation is lethal [12–15]. Therefore, these targets might account for depressed cardiac function in chronic myocardial ischemia.

Thus, we hypothesized that in the human heart alterations in cAMP levels and cardiac Ca\(^{2+}\)-cycling proteins might underlie contractile dysfunction in hibernating and stunned myocardium. Furthermore, putative cardioprotective mechanisms could be activated in human hibernation and stunning. Therefore, in the present study we investigated biochemical parameters known to be altered in end-stage human heart failure and in animal models of hibernation/stunning [3,5,8,10,16].

2. Methods

2.1. Study patients

Patients with coronary artery disease, regional left ventricular dysfunction, clinical indications for elective coronary artery bypass grafting (CABG), areas of myocardial hibernation and/or stunning (target area), as well as normal areas (reference area) were considered eligible to enter the prospective study. Exclusion criteria consisted of myocardial infarction less than 4 weeks prior to CABG surgery, previous cardiac surgery, valvular heart disease, left bundle branch block, diabetes mellitus, and patients with pacemakers, implanted ICD or severe ventricular tachyarrhythmias.

Nineteen patients (17 men, 2 women; age 62±11 years) fulfilled all entry criteria and agreed to participate in the present study. In all patients, coronary revascularization was indicated to improve symptoms (angina) and/or prognosis. All patients had significant coronary artery stenoses (>70%) in vessels that supplied areas of regional left ventricular dysfunction. Prior to surgery, 17 patients (89%) were in New York Heart Association functional classes I or II (elective surgery), but most patients were in CCS angina classes II (n=7), III (n=3), or IV (n=5) (79%). At the time of surgery (biopsy sampling) 8 patients (42%) were on beta blockers (bisoprolol, metoprolol and sotalol) for 795±1152 days (range 75 to 2694 days), 5 patients (26%) were on calcium antagonists (nifedipine, amiodipine, and diltiazem) for 1643±2077 days (range 118 to 5428 days). Written informed consent was obtained from all study patients. The study protocol was approved by the local ethics committee.

2.2. Preoperative evaluation

Detailed preoperative evaluation with respect to regional myocardial function, perfusion, and viability was performed in every patient. This included coronary angiography (anatomy), contrast left ventriculography and two-dimensional echocardiography (resting regional wall motion), single photon emission tomography (SPECT) using \(^{99m}\)Tc-tetrofosmin (myocardial perfusion), and positron emission tomography (PET) using \(^{18}\)F-fluor-deoxy-glucose (FDG) (myocardial viability).

2.2.1. Assessment of regional wall motion

Two experienced investigators, who were blinded to the clinical data, evaluated the preoperative left ventricular cine-ventriculograms and two-dimensional echocardiograms. Regional myocardial function was assessed by segmental evaluation of myocardial wall motion using a semiquantitative wall motion score (normal, hypokinesia, akinesia, dyskinesia). Discrepancies were resolved by consensus.

2.2.2. Myocardial resting perfusion and glucose utilization

Myocardial resting perfusion was measured using single photon emission tomography (SPECT) imaging starting 1 h after injection of 740 MBq \(^{99m}\)Tc-tetrofosmin (Myoview; Amersham Buchler, Braunschweig, Germany) under resting conditions (two heads, 24 projections, \(64\times64\) matrix, \(180°\) orbit, 50 s per step, zoom factor 1.45; Butterworth filter cut-off 0.6, order 5; E.Cam; Siemens Gammasonics Inc., Ill., USA).

Myocardial glucose utilization (MGU) was measured using positron emission tomography (PET) with \(^{18}\)F-fluor-deoxy-glucose (FDG) under hyperinsulinemic, euglycemic steady state conditions [17,18]. The PET scan started >1 h after the onset of the clamp with a rectilinear scan to
position the heart in the field of view, followed by a 20 min transmission scan to correct for attenuation. This was then followed by the measurement of MGU using injection of 370 MBq of the glucose analogue $^{18}$F-FDG and a 23 frames dynamic emission scan ($12 \times 10$ s frames, $4 \times 30$ s frames, $2 \times 300$ s frames, $4 \times 600$ s; $128 \times 128$ matrix; Hanning filter, cutoff 0.5; ECAT EXACT 47 scanner; Siemens/CTI Inc., TN, USA). Parametric images of the regional MGU were calculated according to the Patlak plot, described elsewhere [19,20].

2.3. Intraoperative biopsy sampling

In each patient, transmural left ventricular biopsies were obtained from the dysfunctional target area (hibernation or stunning) and from a reference area with normal myocardial function, perfusion, and metabolism. A biopsy needle permitted sampling of biopsies which were used for Western blotting and cAMP determination.

Areas of intraoperative biopsy sampling were defined and targeted according to preoperative identification of ischemic and dysfunctional, but viable left ventricular myocardium, and of nonischemic reference myocardium, each assessed by coronary angiography, left ventriculography, and other standard clinical parameters (i.e. ECG, stress-echocardiography, $^{99m}$Tc-tetrofosmin-SPECT, $^{18}$F-FDG-PET).

2.3.1. Biopsy localization and image fusion

For comparison of myocardial perfusion and glucose utilization, the $^{99m}$Tc-tetrofosmin-SPECT study and the $^{18}$F-FDG-PET study were matched three-dimensionally using a dedicated multi-purpose imaging tool [21,22]. From these matched data sets, the contour of the left ventricular myocardium was derived using an automated contour finding algorithm, described recently [23,24]. For postoperative co-registration of the biopsy sites with the scintigraphic images, distances from each biopsy position to the apex as well as to the sulcus interventricularis anterior/inferior were measured and recorded during biopsy sampling. On the defined contour of the left ventricular myocardium, the exact position of the biopsy site was then defined by projecting the distances from apex and from the sulcus interventricularis anterior (as seen on the scintigraphic image as the anteroseptal origin of the right ventricle) and sulcus interventricularis posterior (the inferoseptal origin of the right ventricle) on the three-dimensional contour of the left ventricle (Fig. 1). Finally,

Fig. 1. Three-dimensional reconstruction of scintigraphic studies showing relative uptake (% maximum) of $^{99m}$Tc-tetrofosmin (perfusion, left panel) and $^{18}$F-fluor-desoxyglucose (metabolism, right panel) in an inferior view of the heart of one patient. Transmural biopsies were taken from the mid/basal portion of the left ventricular inferior wall, indicated by black circles. These tissue samples exhibit the classical scintigraphic pattern of hibernating myocardium with impaired perfusion and maintained metabolism associated with restoration of wall motion following revascularization [40].
the relative perfusion (PERF) as well as the relative MGU (GLUC) in each biopsy position was derived as compared to the individual segment with the radioactivity maximum in the perfusion study, as described earlier [25]. In parallel, wall motion at each biopsy site was assessed using echocardiography and left ventriculography.

2.3.2. Classification of biopsies

According to the definitions of hibernation and stunning, biopsies were classified into three groups: control, hibernation, and stunning (Table 1). This classification was based on the results of regional wall motion, perfusion and glucose utilization. Biopsies from an area with normal wall motion, perfusion and glucose utilization (PERF, GLUC>70%) were categorized as being control, as previously described with the same methodology [25]. Biopsies from areas of impaired wall motion and reduced resting perfusion (PERF<50%) but with preserved glucose utilization (GLUC>70%) and a significant perfusion/glucose utilization mismatch (GLUC-PERF>20%) were classified as hibernation [25]. Biopsies from dysfunctional areas with normal resting perfusion (PERF>70%) and normal or moderately reduced glucose utilization (GLUC>50%) were categorized as myocardial stunning. All biopsies could be categorized to one of the three groups. Areas with moderate matched reduction of perfusion and metabolism (PERF, GLUC 50–70%), most likely representing non-transmural infarction, as well as areas with transmural scars (PERF, GLUC<50%) were not present in the areas sampled.

2.4. Processing of biopsy specimens

Upon removal, all left ventricular biopsies were immediately freeze-clamped in liquid nitrogen in the operating room. For analysis, biopsy samples were homogenized in teflon vials which were pre-cooled in liquid nitrogen using a microdismembrator (Braun-Melsungen, Melsungen, Germany). An aliquot was taken for the determination of cAMP. The remaining tissues were used for quantitative Western blotting of regulatory proteins in cardiac Ca2+-cycling, Hsp72 and p38 MAP kinase phosphorylation as described [6,8–10].

Table 1

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2.4.1. Adrenergic signal transduction—determination of cAMP

Levels of cAMP were determined in control, hibernating and stunned myocardium by the Biotrak™ cAMP [125I] assay system from Amersham (Amersham Buchler, Braunschweig, Germany) as we have recently reported [8].

2.4.2. Regulatory proteins in cardiac Ca2+-cycling

Western blotting was performed as described earlier [6,8–10]. Phospholamban and SERCA were visualized by enhanced chemifluorescence. CSQ was detected using [125I]-labeled protein A (ICN Biomedicals, Eschwege, Germany).

2.4.3. Stress-activated signal transduction—Hsp72 and p38 MAP kinase

The immunological assay to determine the phosphorylation state of p38 MAP kinase was performed as described recently [10]. Hsp72 was determined by Western blotting as described [9].

2.4.4. Immunohistochemistry

Frozen sections of 5 μm were mounted on silan-coated glass slides and fixed in 4% ice-cold acetone. Sections were incubated for 16 h at 4°C in a humidified chamber with mouse monoclonal antibody against Hsp72 (SPA-810, StressGen, Victoria, Canada) at a dilution of 1:400 in buffer containing 0.6% bovine serum albumin. Bound antibody was detected by rabbit anti-mouse bridging antibody (Dako Diagnostica, Hamburg) and rabbit anti-mouse monoclonal alkaline phosphatase conjugated anti-rabbit immunoglobulin complex (Dianova, Hamburg, Germany). The enzyme reaction was developed in fuchsin solution containing levamisole. Then, sections were counterstained with hematoxyline.

2.5. Statistics

Data are means±standard error of the mean. Statistical analysis was performed to determine statistical differences between hibernating versus control as well as stunned versus control myocardial regions. Except for calsequestrin data, we used the paired Student’s t test, since left ventricular myocardial biopsies were obtained from the same heart. A P value less than 0.05 was considered significant.

3. Results

3.1. Study patients

Complete revascularization was performed on all patients investigated. Biopsy sampling did not cause any complications. Postoperatively, patients significantly improved in NYHA-functional class and CCS angina class.
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* Patients receiving β-blockers (bisoprolol, metoprolol, or sotalol) are indicated with +. Data are given for cAMP (in fmol/mg wet weight), phospholamban (PLB), SR-Ca2⁺-ATPase, calsequestrin (CSQ), inhibitory subunit of troponin (TnI), heat shock protein 72 (Hsp72), phosphorylated p38 (P-p38). Protein expression data are in PhosphorImager units × 10⁻¹⁰. Myocardial perfusion (PERF, ⁹⁹mTc-tetrofosmin) and glucose utilization (GLUC, ¹⁸F-FDG) are expressed as the fraction of the radioactivity in the segment with the maximal uptake in the perfusion study. Wall motion score (WMS): normal (0), hypokinesia (1), akinesia (2), dyskinesia (3). Data are means±standard error of the mean (S.E.M.). *P<0.05, versus control, n: number of patients.
Fig. 2. Myocardial levels of cAMP in human hibernation (A) and stunning (B) compared to control areas. cAMP levels were assayed in transmural biopsies from control (Ctr), hibernating (Hib), and stunned (Stun) human myocardium as described in Methods. Abscissa: experimental group. Ordinate: myocardial cAMP levels in pmol/mg wet weight. Each bar represents the means±S.E.M., the number of n is shown within bars. *P<0.05 vs. Ctr.

(P<0.05; P<0.001 respectively, paired t-test). One patient deteriorated in NYHA-functional class by 1 class, 8 patients improved markedly. While CCS angina class worsened in 1 patient by 1 class, 16 patients improved by up to 3 classes. There was no difference in improvement between patients suffering from stunning or hibernation.

3.2. Biopsy classification

Of the 19 patients included, 10 showed regional hibernation whereas 9 showed stunning. All original scintigraphic and functional data giving the basis of the classification of biopsies into normal, hibernation and stunning are given in Table 2.

3.3. Adrenergic signal transduction—cAMP

Content of cAMP was 30% higher in hibernating myocardium compared to controls (Fig. 2A and Table 2) whereas in stunned myocardium no change of cAMP was noted (Fig. 2B and Table 2).

3.4. Regulatory proteins in cardiac Ca^{2+}-cycling

Next we measured the protein expression of Ca^{2+}-regulatory proteins. Typical autoradiograms of Western blot are shown in Fig. 3. The data in samples from human hibernating, stunned, and control myocardium are summarized in Table 2. No changes in the expression of important proteins for the Ca^{2+}-re-uptake into the sarcoplasmic reticulum, namely phospholamban and SERCA were noted. Furthermore, hibernating and stunned regions showed no differences in the expression of the sarcoplasmic reticulum Ca^{2+}-binding protein CSQ (Table 2). Neither in human hibernation nor in stunning did we notice any significant differences in TnI expression (Table 2).

3.5. Stress-activated signal transduction—Hsp72 and activation of p38 MAP kinase

The expression of Hsp72 was higher by 55% in stunned myocardium but was unchanged in hibernating compared to control myocardium (Fig. 4A and 4B, Table 2). Fig. 5A shows representative immunohistochemistry staining for Hsp72 in control myocardium. Only minor staining for Hsp72 is visible. Fig. 5B (stunned myocardium) reveals more intense labeling (red) for Hsp72 compared to control myocardium.

The phosphorylation state of p38 MAP kinase was unchanged both in hibernating as well as in stunned human myocardium (Table 2).

4. Discussion

The present study demonstrates increased levels of cAMP in human hibernating but not in stunned myocardium. Only in stunned human myocardium was Hsp72 induced.
Fig. 4. Myocardial levels of heat shock protein 72 (Hsp72) in human hibernation (A) and stunning (B) compared to control areas. Hsp72 levels were assayed by Western blotting in transmural biopsies from control (Ctr), hibernating (Hib), and stunned (Stun) human myocardium as described in Methods. Abscissa: group. Ordinate: myocardial Hsp72 levels in PhosphorImager units. Each bar represents the means±S.E.M., the number of n is shown within bars. *P<0.05 vs. Ctr.

increase in the mRNA for heat shock proteins (Hsp 72) and a decline in the mRNA for phospholamban and SERCA [5]. Stunning in a dog model was accompanied by a decrease in cAMP [8]. In patients, only few biochemical data are available (see below).

4.2. Adrenergic signal transduction—β-adrenoceptors

In a very recent investigation biopsies were obtained from both dysfunctional and normal left ventricular regions when patients underwent bypass surgery [11]. These authors reported decreased β-adrenoceptor densities in hibernation and stunning whereas the density of alpha1-adrenoceptors was increased [11]. This decrease in β-adrenoceptor density in hibernation resembles the response that occurs in end-stage human heart failure (reviewed in [16,26,27]). The functional role of increased alpha1-adrenoceptor density remains to be clarified.

4.3. Adrenergic signal transduction—cAMP

A decrease in cAMP content in isolated preparations from failing human hearts has previously been reported [28]. However, these were samples with a different etiology: patients suffered from idiopathic cardiomyopathy requiring cardiac transplantation. Furthermore, in conscious dogs, reductions of cAMP were noted in acute stunning [8]. However, in the present study myocardial levels of cAMP were increased in hibernation but were unchanged in chronic stunning in patients. This would argue for specific biochemical differences (i.e. in the β-adrenergic pathway) in hibernating compared to stunned myocardium as well as in acute versus chronic stunning. Therefore, we here hypothesize that the decrease in β-
Fig. 5. Immunohistochemistry for heat shock protein 72 (Hsp72) in human control (A) and stunned (B) myocardium. Immunostaining was performed in sections from transmural biopsies as described in Methods.
adrenocortical density in hibernation as reported by others [11] is compensated by an increase in cAMP. This could be due to several compensatory mechanisms. These include decreased βARK activity (leading indirectly to an enhanced cAMP formation) [29], increased activity of adenylate cyclase [30], or a reduction in phosphodiesterase (PDE) activity, which would lead to less degradation of cAMP and therefore over time to increased cellular cAMP levels [31]. However, data are currently lacking in order to distinguish between these possibilities in human hibernation and stunning. It is conceivable, that higher intracellular levels of cAMP are necessary to maintain at least the low level of inotropic function observed in the cardiomyocytes. Thus, the observed increase in cAMP could be functionally relevant to maintain responsiveness to β-adrenergic agonists as it occurs during dobutamine challenge in hibernating myocardium.

4.4. Regulatory proteins in cardiac Ca$^{2+}$-cycling

Animal models of hibernation have revealed alterations of phospholamban, CSQ, and SERCA [3,5]. Increased levels in phospholamban reduce contraction [12]. This can be normalized by β-adrenergic stimulation [32]. This is reminiscent of dobutamine challenge in hibernation: depressed cardiac function in hibernation is normalized by dobutamine stimulation of β-adrenoceptors (reviewed in Ref. [33]). In human hibernation, one group reported a decline of phospholamban and an increase in SERCA at protein levels [4]. However, no changes in phospholamban and SERCA expression were measured in the present study. This is probably not surprising as several groups did not note any changes in phospholamban and SERCA (at protein levels) even in terminally failing human hearts (reviewed in Ref. [26]). Moreover, in two porcine models of hibernation phospholamban and SERCA were unchanged [6,7]. While Deindl et al. [4] studied protein levels in only three patients, in the present study hibernating and stunned myocardium from 19 patients was investigated. Within our study, control biopsies from the same hearts were used while Deindl et al. used biopsies from patients with atrial septum defects as controls. The Ca$^{2+}$-binding protein CSQ was increased in a porcine model of stunning [3].

Overexpression of CSQ led to heart failure in transgenic mice [13]. Therefore, in the present study CSQ expression was determined but remained unaltered. In this study, TnI levels were found unchanged and to the best of our knowledge, this is the first report on TnI expression levels in human hibernating and stunned myocardium. However, decreased expression of TnI occurred in isolated cardiac models of stunning [34,35]. In intact animals no changes in TnI levels were noted, neither in hibernation nor in stunning [6–8,10]. Hence, the lack of changes in TnI in the present study is in line with previous animal experiments from independent groups [6–8,10]. Recently, in an animal model of hibernation for 24 h a decrease in Ca$^{2+}$ sensitivity of the myofilaments was noted [36].

4.5. Stress-activated signal transduction—Hsp72 and activation of p38 MAP kinase

Both in animal models of chronic hibernation and acute stunning the expression of Hsp72 was increased [5,9]. Increased cardiac expression of Hsp72 protects hearts of transgenic mice against cardiac dysfunction from ischemia [37].

In the present study, Hsp72 was not increased in chronic hibernation in the human heart. This can be explained by the fact that ischemia alone is not a sufficient trigger for Hsp72 induction. Only ischemia followed by reperfusion is known to induce Hsp72 in animal models [38]. Furthermore, in human hearts after bypass surgery, increased levels of Hsp72 have been reported in reperfused myocardium [39].

Hsp72 could exert cardioprotective effects in human stunning [40]. Furthermore, an animal model of acute hibernation has revealed a transient increase in the activation of the stress-activated p38 MAP kinase during moderate low-flow ischemia [10]. However, p38 MAP kinase activation was unchanged in hibernating or stunned human myocardium. This is compatible with the idea that sustained p38 MAP kinase activation would cause damage as it occurs in terminal heart failure due to ischemic cardiomyopathy. Fittingly, in this disease p38 MAP kinase was activated [41]. However, in the present study hibernating and stunned myocardial biopsies were viable and p38 activation was absent.

4.6. Myocardial blood flow in hibernating myocardium

The pathophysiological concept of hibernating myocardium concerning the myocardial blood flow is still controversially discussed. Hypotheses include the concept of chronic severe ischemia vs. repetitive stunning. Initially, non-invasive imaging including myocardial scintigraphy showed a typical mismatch between impaired regional perfusion and normal or enhanced metabolism ($^{18}$F-FDG-PET) in hypokinetic areas, a phenomenon that was found to be predictive of restoration of wall motion following revascularization [25]. This mismatch was assumed to reflect myocardial hibernation. However, recent studies using quantitative measures of myocardial perfusion ($^{15}$O-PET, $^{13}$N-ammonia-PET) demonstrated normal or only mildly impaired resting perfusion in hibernating myocardium, which in principle supports the hypothesis of repetitive stunning. This could be attributed to different facts, e.g. that with PET-technology one can measure perfusion in the fraction of a tissue region which is perfusable (perfusable tissue index PTI, excluding scar tissue) and that the uptake of these tracers is more independent (esp. $^{15}$O) of the viability of the myocytes as it is with the SPECT tracers such as tetrofosmin.
However, the technology used in this study ($^{99m}$Tc-tetrofosmin, $^{18}$F-FDG-PET) is still the clinical ‘gold standard’ in preoperatively predicting viability associated with restoration of wall motion following revascularization. This is extensively discussed elsewhere [42,43].

5. Limitations of the study

In the present study the medication varied among patients. However, control myocardium was obtained from each patient. Thus, individual medication should have influenced control myocardium to the same degree as hibernating or stunned myocardium, respectively. If, on the other hand, β-adrenergic activation of stunned or hibernating myocardium exceeded that of control myocardium in the same subject, it is conceivable that the medications may have had a greater impact on the stunned or hibernating myocardium than on the control area.

The scintigraphic classification of hibernating myocardium used in the present study follows standard and well-established criteria [25]. In contrast, there is no standard scintigraphic classification of stunning. Therefore, the classification of stunning used in this study was derived from the pathophysiological concept of postischemic myocardial dysfunction (prolonged dysfunction, normal perfusion, normal or impaired metabolism). Myocardial perfusion was assessed by SPECT studies with the inherent limitations of potential attenuation artifacts. These artifacts lead to an underestimation of the real radioactivity by about 10% in typical locations (e.g. inferior wall) in only some patients. This would result in an artificial mismatch of perfusion and metabolism. However, the typically huge difference between impaired perfusion and preserved metabolism in the areas classified as hibernating myocardium in this study cannot be explained as being caused by attenuation artifacts. At the time of the study, perfusion PET with attenuation correction was not available at our center (FDG-satellite concept).

6. Conclusions

In conclusion, the increased myocardial cAMP content in human hibernation might maintain contractile responsiveness upon adrenergic stimulation despite diminished β-adrenoceptor density. In human stunned myocardium increased Hsp72 content could have cardioprotective effects. However, changes in the expression of Ca$^{2+}$-handling proteins do not underlie the contractile dysfunction in human hibernating and stunned myocardium.

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