Insulin-like growth factor-II delays myocardial infarction in experimental coronary artery occlusion

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Abstract

Objective: We have previously shown that short pulses of myocardial ischemia cause increased mRNA expression of the insulin-like growth factor II (IGF-II) gene. The expression of IGF-II precedes the expression of its binding protein 5 (IGFBP-5). The cardioprotective actions of the IGF-II peptide and of its binding protein 5 as well as the underlying mechanisms were investigated in this study. Methods and results: Human recombinant IGF-II 0.25 mg/ml was applied by means of direct intramyocardial infusion (IM) for 60 min prior to a 60 min LAD occlusion and 120 min reperfusion. Myocardial infarction, compared to the region at risk, was significantly decreased by IGF-II treatment, whereas infusion of Krebs-Henseleit buffer did not show any protective effect (IGF-II, 78.75 ± 1.51%; control, 100%; P < 0.005). A comparable degree of cardioprotection was observed after infusion of an equipotent concentration of recombinant human insulin (0.02 IU/ml; 88.25 ± 1.45%; P < 0.05). Lavendustin A (100 μM), an inhibitor of protein tyrosine kinases, prevented the observed cardioprotection. The protective effect of IGF-II was lost when IGFBP-5 was simultaneously infused. Conclusion: IGF-II, a peptide that binds to the insulin receptor and whose mRNA is rapidly transcribed by cardiac myocytes following ischemic stress, is cardioprotective and mimics ischemic preconditioning. Its observed actions are probably based on its metabolic effects and are mediated by the insulin or the IGF-I receptor. IGFBP-5, whose expression follows IGF-II’s expression with a short delay, inhibits the cardioprotection afforded by IGF-II and may thus account for the limited temporal duration of ischemic preconditioning.

Keywords: Insulin-like growth factor II; Insulin; Myocardial protection; Preconditioning; Myocardial infarction

1. Introduction

Repetitive coronary occlusions of short duration render the heart resistant against a subsequent long period of ischemia. This experimental intervention, termed ‘ischemic preconditioning’ [1], is the most powerful protection against myocardial infarction for the in situ beating heart.

Previous work from our laboratory had demonstrated significant changes in myocardial gene expression in myocardium subjected to ischemic preconditioning [2]. Amongst other genes, insulin-like growth factor II (IGF-II) and insulin-like growth factor binding protein 5 (IGFBP-5) [3] were upregulated by ischemia/reperfusion but also by surgical stress. The insulin-/insulin-like growth factor system is an endocrine/paracrine physiologic system controlling metabolism, growth, differentiation, and the response to stress (Fig. 1) [4,5]. Recent observations in neuronal models suggest trophic and protective actions of the IGFs in the setting of ischemia and reperfusion [1,6–9]. Like adult myocytes, neurons are terminally differentiated cells that exhibit pathways of endogenous protection similar to ischemic preconditioning in the heart [10–14]. In skeletal muscle, it was demonstrated that the IGFs stimulate glucose uptake by their insulin-like activity [15,16]. It is therefore tempting to speculate that in times of metabolic stress, as in ischemia, the heart expresses ‘its own insulin’ by making use of the metabolic properties of IGF-II. Since the expression of IGF-II is followed by the expression of one of its binding proteins which terminates its action, we hypothesize that ischemic preconditioning can, at least in part, be explained on the basis of changes of the IGF tissue concentration.
Since the appearance of protein may follow the increased mRNA content with only a short delay [17], the aim of the present study was to investigate whether the increased expressions of IGF-II and its binding protein 5 may be operative in increasing myocardial resistance against infarction.

To test its cardioprotective actions, human recombinant IGF-II was directly infused into porcine myocardium prior to coronary occlusion and reperfusion normally causing myocardial infarction. To further elucidate the underlying mechanism, an equipotent dose concerning IGF-II’s metabolic effects of recombinant human insulin was tested in a second group. The receptors involved were further characterized by simultaneous infusion of lavendustin A to block the protein tyrosine kinase domain of either the IGF-I or the insulin receptor [18,19].

There is very little knowledge available yet about the physiologic functions of the IGF-binding proteins. Recent investigations have demonstrated that the physiologic actions of the IGFs (i.e., cellular glucose uptake) can be inhibited by their binding proteins [16,20]. In our porcine model of repeated short coronary occlusions, the expression of IGFBP-5 follows the expression of IGF-II with a short delay [1,3]. Due to the good correlations of the time courses of the appearance of IGFBP-5 mRNA and the vanishing protection of ischemic preconditioning [21] we hypothesized that the increased content of IGFBP-5 with prolonged reperfusion periods will inhibit IGF-II’s cardioprotective actions. Possible interference of the expression of IGFBP-5 was investigated by a simultaneous infusion of IGFBP-5 with IGF-II prior to ischemia and reperfusion.

2. Methods

The experimental protocol described in this study was approved by the Bioethical Committee of the District of Darmstadt, Germany. Furthermore, all animals in this study were handled in accordance with the guiding principles in care and use of animals as approved by the American Physiological Society and the investigation conformed with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.1. Animal preparation

Sixteen castrated male German landrace-type domestic pigs with body weights between 27.5 and 35.5 kg (32.6 ± 0.22 kg) were premedicated with 2 mg·kg⁻¹ BW i.m. azaperone (Stresnil, Janssen Pharmaceutica, Neuss, Germany) and 2 mg·kg⁻¹ BW s.c. piritramid (Dipidolor, Janssen Pharmaceutica, Neuss, Germany) 30 min prior to the initiation of the anesthesia with 10 mg·kg⁻¹ BW metomidate (Hypnodil, Janssen Pharmaceutica, Neuss, Germany). After either oral or endotracheal intubation, a bolus of 25 mg·kg⁻¹ BW of α-chloralose (Sigma, Deisenhofen, Germany) was given intravenously. Anesthe-
sia was maintained by a continuous intravenous infusion of 25 mg·kg⁻¹ BW·h⁻¹ α-chloralose. The animals were ventilated artificially with a pressure-controlled respirator (Stephan Respirator ABV, F. Stephan GmbH, Quickborn, Germany) with room air enriched with 2 l·min⁻¹ oxygen. Arterial blood gases were analyzed frequently to guide adjustment of the respirator settings. Additional doses of piritramid (10 mg) were given i.v. every 60 min.

Both internal jugular veins were cannulated with polyethylene tubes for administration of saline, piritramid and α-chloralose. Arterial sheath catheters (7F) were inserted into both common carotid arteries. To measure aortic blood pressure, the left sheath was advanced into the aortic arch and connected with a Statham transducer (P23XL, Statham, Puerto Rico). A 5F high-fidelity catheter-tipped manometer (Millar Instruments, Houston, TX, USA) was inserted via the right common carotid artery into the left ventricle to measure left ventricular pressure and to calculate its first derivative (LV dP/dt). The chest was opened by a midsternal thoracotomy and the heart was suspended in a pericardial cradle. A loose silk ligature was placed halfway around the left anterior descending coronary artery (LAD), and was subsequently tightened to occlude the vessel. After preparation, a stabilization period of 20 min was allowed and the different experimental protocols were started.

Index ischemia was a 60 min LAD occlusion followed by 120 min reperfusion. If ventricular fibrillation occurred, a defibrillator with paddles placed onto the external chest wall was used for conversion. Data from DC-amplifiers including surface ECG (Heinemann, Frankfurt/Main, Germany) were digitized by an analog-digital converter (MacLab/8, AD Instruments Pty Ltd., Castle Hill, Australia) and continuously recorded onto the hard disc of a personal computer (Power Book 180, Apple Computer Inc., Cupertino, CA, USA). Body temperature was maintained within the physiological range by thermal isolation of the animals. Of the 16 animals investigated, 5 experienced ventricular fibrillation during LAD occlusion and were successfully defibrillated. To avoid the side-effects of systemic injections of either IGF-II or insulin on systemic glucose metabolism [22] we treated the myocardium of interest only locally by intramyocardial infusion as described in detail previously [23,24].

Eight 26-gauge needles with a free length of 5 mm that were connected by tubing with a peristaltic pump (Minipuls, Gilson, Germany) were placed in pairs into the subsequently ischemic myocardium perpendicular to the epicardial surface. The substances were infused at 20 µl/min/needle, amounts far below those causing distension of the myocardium.

2.2. Experimental groups

The experimental design is demonstrated in Fig. 2. Five groups of animals were studied (in a sixth group of animals a different design and methodology was used).
Fig. 1. Signal transduction pathway of the insulin/IGF system. Insulin, IGF-I and IGF-II bind to their own receptors with high (bold arrows) and to the other receptors with lower (thin arrows) affinity. Insulin does not bind to the IGF-II receptor. The insulin and the IGF-I receptor are homodimers consisting of extracellular (α), transmembrane and intracellular domains (β). The intracellular domains have protein tyrosine kinase activity that can be blocked with lavendustin A. In general, the insulin receptor mediates metabolic effects whereas anabolic and proliferative effects are transmitted by the IGF-I receptor. The IGF-II receptor that was not detectable in cardiac tissue by Northern blot analysis [3] is a G-protein-coupled receptor. Its exact function remains to be elucidated.

Fig. 2. Experimental groups. Six experimental groups were investigated. After stabilization (20 min), groups 1 to 3 consisted of animals receiving microinfusions of Krebs-Henseleit buffer, IGF-II, or insulin respectively. The animals of groups 4 and 5 were treated with simultaneous infusions of IGF-II/insulin and lavendustin A. Group 6 was subjected to microinfusions of IGF-II and IGFBP-5. In all animals, index ischemia causing myocardial infarction was 1 h LAD occlusion followed by 2 h of reperfusion.
In all animals index ischemia was a 60 min LAD occlusion followed by 120 min reperfusion. The control group (1) consisted of animals receiving intramyocardial infusions of Krebs-Henseleit buffer for 60 min prior to index ischemia. In group 2, recombinant human IGF-II (250 ng/ml, Biomol, Hamburg, Germany) was infused for 60 min. The animals of group 3 received infusions of recombinant human insulin (0.02 IU/ml, H-insulin Hoechst®, Hoechst AG, Frankfurt, Germany) in an equipotent concentration with regard to IGF-II’s metabolic effects. In groups 4 and 5, either IGF-II or insulin were co-infused with lavendustin A (100 μM), a potent and selective inhibitor of protein tyrosine kinases [25]. To make sure that protein tyrosine kinases were inhibited at the onset of IGF-II/insulin infusion, lavendustin A was infused alone 30 min before adding the other substances.

To test the expected inhibitory action of IGFBP-5, the animals of group 6 received co-infusions of IGF-II and recombinant human IGFBP-5 (Austral Biologicals, San Ramon, CA, USA) in equimolar concentrations.

The concentrations used were determined in previous pilot experiments. Concerning IGF-II’s cardioprotective actions, a dose–response testing indicated no protection at pilot experiments. Concerning IGF-II’s cardioprotective effects, in groups 4 and 5, either IGF-II or insulin were co-infused with lavendustin A 100 μM, a potent and selective inhibitor of protein tyrosine kinases [25]. To make sure that protein tyrosine kinases were inhibited at the onset of IGF-II/insulin infusion, lavendustin A was infused alone 30 min before adding the other substances.

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The concentrations used were determined in previous pilot experiments. Concerning IGF-II’s cardioprotective actions, a dose–response testing indicated no protection at the concentrations of 2.5 and 25 ng/ml. At 50 ng/ml, a small, inconsistent effect was observed whereas 250 ng/ml showed reproducible, marked protective effects. IGF-II stimulates metabolic insulin effects with a potency of about 25% of the action of insulin itself [15,26]. For this reason we have chosen the insulin concentration as described above (i.e., 25% of IGF-II’s molar concentration).

IGFBP-5, a protein showing two bands at 31 and 32 kD in SDS-PAGE [27], was tested in concentrations of 50, 100 and 200% relative to IGF-II’s molar concentration. The cardioprotective effect of IGF-II was inhibited at equimolar concentrations and when IGFBP-5 was co-infused. Since IGFBP-5 shows no affinity to insulin [28], this experimental situation was not tested.

2.3. Exclusion criteria

Perfusion sites were excluded from evaluation if systolic-diastolic cardiac movements caused dislocation of microinfusion needles or if protected areas were not totally surrounded by PNBT-negative, necrotic tissue. Successful DC countershock treatment of ventricular fibrillation was not a criterion for exclusion. Since intramyocardial infusion was finished before the onset of index ischemia, a possible dislocation of the microinfusion needles at this time point could not interfere with the result and was hence not an exclusion criterion.

2.4. Measurement of infarct size

At the end of the experimental protocol, the left anterior descending coronary artery was occluded again and 1 g fluorescein-sodium (Fluka, Neu-Ulm, Germany, dissolved in 10 ml saline) was injected as a bolus intravenously. After the overall distribution of the dye the animals were sacrificed with an intravenous bolus of 20% potassium chloride (30 ml) to achieve cardiac arrest. The heart was excised and both atria and the right ventricle were removed. The left ventricle was cut into slices along the pairwise inserted microinfusion needles perpendicular to the LAD. After weighing the slices, the ischemic area was identified as the non-fluorescent area by blacklight examination. The infarcted area was demarcated using p-nitro-blue-tetrazolium (pNBT) staining. The results of the stainings were documented on transparency film for further planimetric evaluation. Due to the onset of rigor mortis the heart slices sometimes shrink between drawing of the fluorescein outlines and the tetrazolium incubation, which makes it sometimes difficult to match the outlines of the risk region with that of the infarct. Since the fluorescein leaks out during incubation, it is impossible to determine the risk region and the infarct size after the tetrazolium bath. We have therefore replaced in some animals the fluorescein method with fluorescent microspheres that do not leak out during incubation. The color transparencies of the risk region and of the infarcts were scanned into an Apple Quadra computer and a composite image was created using ‘Photoshop’ graphics software.

2.5. Planimetry and quantification of myocardial protection

Since only a fraction of the ischemic myocardium was reached by the infused substances, quantification of myocardial protection was done as follows:

Myocardial infarcts in the pig after 60 min coronary occlusion and 120 min reperfusion are homogenous and transmural. Fig. 3 demonstrates that the substances were infused into a region that, according to our experience, becomes necrotic afterwards. Intramyocardial infusions of fluorescein sodium (done in several animals) helped us to identify the perfused myocardial areas, as determined by blacklight examination. PNBT-positive (viable) myocardial areas in the tissue close to the needles (3b), totally surrounded by PNBT-negative (necrotic) tissue, were regarded as myocardial areas protected by intramyocardial infusion. Description and statistical evaluation of myocardial protection was done in two ways as follows. Using a nominal scale, evaluation was done by differentiating between the infusion sites showing ‘protection’ and ‘no protection’. These results are shown in Table 1. The other method consisted of comparing the infarcted areas by planimetry: The border zones between ischemic and non-ischemic myocardium were not reached by our microinfusion technique, as determined by dye infusions (3a). Thus, we made sure that the outline of the infarct remained unaffected by the active compounds. In this way, every myocardial slice planimetred served as its own control:
Fig. 3. Intramyocardial microinfusion. The microinfusion needles were placed in pairs into the LAD territory that will become ischemic afterwards (3a). Tissue distribution (as determined by dye infusions) indicated by dark areas. After pNBT staining to detect myocardial infarction (lower row), myocardial protection was seen by pNBT-positive, viable tissue surrounding the needles (striped areas in 3b) which did not occur if the infused substances were without protective effect. Myocardial protection was expressed by relating the actual infarcted area (white area in 3b, left) to the infarcted area that would have become necrotic assuming transmural infarction (white area in 3b, right).

First, we determined the infarcted area that would have occurred without protection (i.e., the total area of the transmural infarction). This area was set as 100%. In the second step, we determined the infarcted area (transmural area minus protected myocardial area) and related this value to the area obtained in step one. Per animal and substance, one mean ratio in percent risk region was determined and subjected to further statistical evaluation.

2.6. RNA isolation and Northern hybridization

Six additional animals were anesthetized, intubated and artificially ventilated as described. In 3 animals a PTCA catheter was advanced into the left anterior descending coronary artery that was occluded by balloon inflation for 10 min. Catheter inflation was performed at pressures that occluded but did not stretch the arteries. Occlusion was checked by fluoroscopy and contrast medium injection. The catheter was thereafter deflated and withdrawn for 30 min and again advanced under fluoroscopic guidance into the LAD for another round of 10 min occlusion and 30 min of reperfusion. Thereafter the animal was sacrificed by an overdose of the anesthetic plus a bolus of concentrated KCl. The heart was removed and quickly dissected into the ischemic-reperfused LAD region and the normal left ventricle. In 3 other pigs only the arterial cutdown was performed and the hearts were removed after 80 min of anesthesia, an identical amount of contrast medium injection and after sacrifice with nembutal and KCl. These hearts served as controls; they were dissected in an identical way to that of the PTCA hearts.

RNA extraction, electrophoresis and cDNA probe fashioning were done according to standard protocols and as previously described [3,29,30]. Quantification and referencing of the Northern signal were carried out by PhosphorImager as previously described [3,29,30].

2.7. Statistical analysis

All data are given as mean values with standard error of mean. Statistical comparisons between groups were done by ANOVA (Scheffé test) unless mentioned otherwise. A P-value smaller than 0.05 was considered statistically significant.

3. Results

3.1. Hemodynamic data

Fig. 4 shows the hemodynamic data recorded throughout the experiments. Compared to baseline values (t = 0 min), intramyocardial microinfusion for 60 min did not affect systemic hemodynamics. The time course of hemodynamics during ischemia and reperfusion follows the

Fig. 4. Hemodynamic data. Systemic hemodynamic data from all experiments shown as mean±standard error of mean. Minutes 30 to 60: intramyocardial microinfusion. Minutes 61 to 120: LAD occlusion. Minutes 121 to 240: reperfusion. LVP = left ventricular pressure; AOP = aortic pressure; dP/dt = first derivative of left ventricular pressure; s = systolic; d = diastolic; HR = heart rate.
3.2. Myocardial protection

Table 1 demonstrates myocardial protection achieved in the different experimental groups. Statistical analysis of the data by contingency table analysis shows that the $P$-value would be $> 0.001$ in the case of one dropout (i.e., a change in the wrong direction). Since we had no dropouts, the $P$-value would be even smaller. IGF-II as well as insulin displayed cardioprotection at every infusion site investigated ($n = 16$) in 4 animals. These effects were inhibited by the simultaneous infusion of lavendustin A, an inhibitor of protein tyrosine kinases, blocking the signal transduction of IGF-II and insulin at the receptor level. Likewise, simultaneous infusion of IGFBP-5 in all experiments prevented protection by IGF-II.
Table 1
Myocardial protection in the different experimental groups using a nominal scale

<table>
<thead>
<tr>
<th>Group</th>
<th>Protection</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>IGF</td>
<td>16/4</td>
</tr>
<tr>
<td>INS</td>
<td>16/4</td>
</tr>
<tr>
<td>IGF + LAV</td>
<td>0</td>
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<tr>
<td>INS + LAV</td>
<td>0</td>
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<tr>
<td>IGF + BP5</td>
<td>0</td>
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The first number indicates the slices evaluated, the second number the animals investigated. For further explanation, see text.

Fig. 5 demonstrates that the data for areas at risk did not differ significantly in the groups investigated. The conventional way of expressing infarct size is shown in Fig. 6. An example of infarct imaging is given in Fig. 7.

3.3. IGF-II expression

IGF-II mRNA is 4-fold overexpressed following coronary occlusion by balloon inflation in two animals (see Fig. 8). The third balloon-occluded animal died of ventricular fibrillation. The data of 2 control animals are not shown because no increased IGF mRNA expression was noted. In 1 control animal the RNA was degraded. No statistical analysis was attempted because of the small number of animals and the large differences in mRNA expression between balloon-occluded and control tissue.

4. Discussion

In a previous paper [3] we described increased and very fast IGF-II mRNA expression following repeated brief ischemia under open-chest conditions in the pig. Since the expression of the IGF binding protein 5, which interferes with the action of IGF-II, was also upregulated, albeit with a phase lag of about 30 min, we hypothesized that the protection caused by ischemic preconditioning can also be explained by the action of the IGF system where protection is initiated by IGF-II and terminated by IGFBP-5. Since this hypothesis cannot be proven by mRNA data alone and since available antibodies directed at human recombinant IGF-II do not cross-react with the porcine homolog, we tested this idea by direct intramyocardial infusion of the peptides involved. Our results show that indeed IGF-II delays the progression of an experimental infarct just like ischemic preconditioning and IGFBP-5 neutralizes this effect. We show further that the protective effect is mediated via the insulin receptor which belongs to the tyrosine kinase class of receptors and that the effect can be neutralized by the tyrosine kinase inhibitor lavendustin. IGF-II binds either to the IGF-I receptor, the IGF-II/mannose 6-phosphate or to the insulin receptor and has hence either a mitogenic or a metabolic effect. Our results suggest that the cardioprotection is based on the metabolic effects of IGF-II because:

- insulin itself produced a similar degree of protection,
- insulin does not bind to the IGF-II and only weakly to the IGF-I receptor,
- neither the IGF-I nor the IGF-II receptor produces a metabolic effect [5,32] and
- the IGF-II receptor is not expressed under the experimental conditions that we have created [33], which is compatible with the low number of IGF-II receptors reported for the adult heart [1,34]. The mRNAs of the insulin and the IGF-I receptor, on the other hand, are abundantly expressed in porcine heart.

To the best of our knowledge, this is the first report describing cardioprotection by one of the insulin-like growth factors. In contrast to the heart, the role of the IGF’s is better investigated in neuronal models. Recent investigations reported a beneficial role for the IGF’s in brain ischemia [6,9], and the effect was also produced by its metabolic effects [6,7]. In the heart, influencing glucose metabolism has been shown to be beneficial in ischemia/reperfusion settings [35–37] and our preliminary experiments with positron emission tomography (together with Schwaiger, unpublished) showed increased focal glucose uptake after intramyocardial IGF-II infusion, from which we conclude that this was one of the underlying mechanisms responsible for the increased ischemia resistance of the myocardium [15,16]. Interestingly, increased myocardial glucose uptake is a characteristic feature also of ‘hibernating myocardium’ [1,38] to protect itself from sustained ischemic stress [39]. Thus, the increased mRNA content for IGF-II may be interpreted to take place as a self-defense mechanism of myocardium subjected to repeated episodes of ischemia. Thereby the heart circumvents its inability to synthesize insulin itself. Instead, it increases its production of insulin-like growth factor II, ‘the heart’s own insulin’, to increase its tolerance against subsequent ischemic episodes.

Since IGF-II mRNA is markedly overexpressed in preconditioned porcine myocardium and IGF-II peptide was demonstrated to be protective in the setting of cardiac ischemia and reperfusion, an involvement of IGF-II in preconditioning’s cardioprotection appears likely since the translation of small-peptide hormones may happen quickly, even in ischemia [17]. The delay that is necessary for ischemic preconditioning to become manifest may correspond to the time necessary to translate IGF-II mRNA. Although recent reports in the rabbit suggest ischemic preconditioning to be independent of de novo protein synthesis [40], the conclusion rested on the assumption that the dose of cycloheximide was sufficient to inhibit global protein synthesis. This may not apply to small peptides like the IGFs. The 30 min delay with which IGFBP-5 mRNA expression follows the expression of IGF-II [3] could explain why the memory effect of ischemic precon-
ditioning (i.e., the fact that it ‘remembers’ short occlusions) is ‘forgotten’ after less than 1 hour following the last conditioning short occlusion and that cardioprotection is no longer present after that time [21]. By extending the time period after the repetitive coronary occlusions the increase in IGFBP-5 content will terminate preconditioning’s cardioprotection: i.e., the protection observed is the resultant of the myocardial content of IGF-II (protective) and IGFBP-5 (inhibitory).

This proposed mechanism explains the observation that in our porcine model ischemic preconditioning is not yet optimal when shorter protocols of repetitive coronary occlusions [41] are used (2 occlusions of 10 min or 4 occlusions of 5 min are needed for protection, but only the first produced significant amounts of adenosine) in contrast to other species [24,42]. This would suggest that the adenosine hypothesis in the porcine heart can only partially explain the protective effect of short occlusions and that an additional factor has to be assumed. This is most likely the IGF system: the multiple occlusions needed for protection in our model produce the necessary amounts of IGF-II. The functional synergism between adenosine and insulin with regard to glucose uptake that is stimulated by both agents is indeed very helpful for cytoprotection [43,44]. Although it was shown that neither insulin nor adenosine increase glucose uptake during prolonged ischemia, the reperfusion periods in between brief occlusions may stimulate glucose uptake. Other properties of IGF-II-like inhibition of protein breakdown and preservation of translation may add to the cytoprotective profile of the hormone [45].

One argument encountered during the review of our previous paper was that the upregulation of IGF-II mRNA expression may not be specific for ischemia but may also have been caused by the surgical stress of the open-chest situation, because a statistically non-significant trend seemed to indicate that. Furthermore, there was no difference in IGF-mRNA between ischemic-reperfused and adjacent non-ischemic myocardium. This argument can now be placed into proper perspective with our new data that show upregulation of IGF-II mRNA expression in a closed-chest model only with ischemia and in the ischemic region (see Fig. 8). We conclude from these and from the previous findings that both surgical stress and ischemia proper are able to upregulate IGF-II mRNA. On the basis of mRNA data alone it cannot be inferred that IGF-II may have a protective effect because it could have been an epiphenomenon related to stress. However, our direct infusion studies show clearly the protective potency of the hormone.

In summary, our data describe a cardioprotective effect of IGF-II, a tissue hormone with insulin-like actions whose mRNA is 4- to 5-fold overexpressed in preconditioned myocardium. The cardioprotection caused by IGF-II is mediated via the insulin receptor. IGFBP-5, whose expression follows the expression of IGF with a short delay, inhibits the cardioprotection afforded by IGF-II and thereby possibly accounts for the limited protective time frame of ischemic preconditioning. IGF-II and adenosine cooperate by increasing the glucose uptake in ischemic-reperfused myocardium and they may act via translocation of the glucose transporter.

4.1. Limitations of the study

The method of direct infusion of tool drugs into the myocardium was developed for the study of focal arrhythmias [46]. We show here and in a previous paper [24] that it is also useful for the application of hormones that act mainly locally and that cannot be applied systemically in large animals due to cost and to serious side-effects. The disadvantage of the method is that the concentration of the hormone at some distance from the infusion needle is not known. With continuous infusion the gradients of concentration will decrease, but the final space of distribution remains unknown. Experiments with dyes showed that at a rate of infusion of 20 μl/min a volume of approximately 1 cm³ is reached within 10 min. Longer infusion times increase the volume of distribution, but we do not at present know where the saturation point is.

References


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