Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 470 (2003) 177–183
www.elsevier.com/locate/ejphar

The administration of α-melanocyte-stimulating hormone protects the ischemic/reperfused myocardium

Miklos Vecsernyes, Bela Juhasz, Peter Der, Reka Kocsan, Palma Feher, Ildiko Bacskay, Peter Kovacs, Arpad Tosaki*

Department of Pharmacology and Clinical Pharmacology, Health and Science Center, School of Medicine, University of Debrecen, Nagyerdei krt. 98, 4032 Debrecen, Hungary

Received 6 February 2003; received in revised form 15 April 2003; accepted 25 April 2003

Abstract

The contribution of α-melanocyte-stimulating hormone (α-MSH) treatment, an active fragment of adrenocorticotropic hormone (ACTH), to the recovery of postischemic cardiac function, infarct size, the incidence of reperfusion-induced ventricular fibrillation and apoptotic cell death was studied in ischemic/reperfused isolated rat hearts. Rats were subcutaneously injected with 40, 200 and 400 μg/kg of α-MSH, and 12 h later, hearts were isolated, perfused and subjected to 30 min of ischemia followed by 120 min of reperfusion. Thus, after 120 min of reperfusion, with the concentration of 200 μg/kg α-MSH, coronary flow, aortic flow and left ventricular developed pressure were significantly improved from their control values of 14.6 ± 0.6 ml/min, 7.5 ± 0.5 ml/min and 9.1 ± 0.4 kPa to 20.2 ± 0.4 ml/min ($p < 0.05$), 31.5 ± 0.9 ml/min ($p < 0.05$) and 15.9 ± 0.6 ($p < 0.05$) kPa, respectively. With the doses of 40, 200 and 400 μg/kg of α-MSH, infarct size was reduced from its control value of 38 ± 5% to 33 ± 6% (NS), 17 ± 3% ($p < 0.05$) and 19 ± 4% ($p < 0.05$), respectively. The reduction in the incidence of reperfusion-induced ventricular fibrillation followed the same pattern. It is reasonable to assume that a reduction in infarct size, in the α-MSH-treated myocardium, resulted in a reduction as well in apoptotic cell death. Although we did not specifically study the exact mechanism(s) of α-MSH-afforded postischemic protection, we assume that this protection may be related to α-MSH-induced corticosterone release and corticosterone-induced de novo protein synthesis, which reflected in the recovery of postischemic cardiac function in isolated hearts. Thus, interventions that are able to increase plasma corticosterone or glucocorticoid release may prevent the development of ischemia/reperfusion-induced damage.

Keywords: α-MSH (melanocyte-stimulating hormone); Ischemia; Reperfusion; Corticosterone; Heart, rat

1. Introduction

Myocardial reperfusion by transluminal angioplasty, thrombolytic therapy and bypass surgery has emerged the fundamental strategy in the management of acute ischemic episodes. Furthermore, it is well known that spontaneous reperfusion after coronary artery spasm or thrombosis is a common event in humans with coronary artery diseases. In the past three decades, intensive research was done, and the results confirmed that reperfusion, restoration of flow, nutrition and oxygen to the previously ischemic tissue triggers sudden metabolic, electrophysiological, morphological and functional changes (Hearse et al., 1978; Hearse and Bolli, 1992). The mechanisms underlying the genesis of ischemia/reperfusion-induced myocardial damages are complex and not clearly understood. It is highly possible that a number of interacting mechanisms combine to determine the extent of reperfusion-induced damage including α and β receptors (Sheridan et al., 1980), ions (Curtis et al., 1993; Opie, 1991) and their exchangers (Avkiran et al., 2001), fatty acids and phospholipids (De Groot et al., 1993; Van der Vusse et al., 1994), free radicals (Bolli, 1988), various gene regulations (Kolbeck-Ruhmkorff and Zimmer, 1995; Keating and Sanguinetti, 2001; Simkovich et al., 2002) and apoptotic signals (Maulik et al., 1998; Suzuki et al., 2002). Recently, the concept has been introduced that α-melanocyte-stimulating hormone (α-MSH) may protect renal cells and tissues against ischemia/reperfusion-induced injury (Chiao et al., 1997). α-MSH (1–13 amino acids) is an active fragment of adrenocorticotropic hormone (ACTH, 1–

* Corresponding author. Tel./fax: +36-52-427899.
E-mail address: tosaki@king.pharmacol.dote.hu (A. Tosaki).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reseved.
doi:10.1016/S0014-2999(03)01780-1

39 amino acids), and it has been reported that ACTH and its fragments (in various degree) including α-MSH have a prompt and sustained resuscitating effect in conditions of severe tissue hypoxia, either due to hypoperfusion including hemorrhage shock (Bertolini et al., 1986, 1987), cardiogenic shock (Noera et al., 1991), splanchnic artery occlusion-induced shock (Squadrito et al., 1999) or to prolonged respiratory arrest. Relatively little attention, to our knowledge, has been paid on the effects of ACTH and its fragments, especially α-MSH, on the recovery of postischemic cardiac function. However, it is of interest to note the finding of Bazzani et al. (2001) studying the antiarrhythmic and antiapoptotic effects of ACTH and [Nle^4, D-Phe^7]α-melanocyte-stimulating hormone. These authors (Bazzani et al., 2001) showed, for the first time, that melanocortin peptides significantly reduced the incidence of ventricular tachycardia and ventricular fibrillation, ischemic area and apoptotic cell death under in vivo conditions in a rat model of cardiac ischemia/reperfusion. The aim of our study was not to analyze what is the action mechanism(s) of α-MSH to protect ischemic/reperfused myocardium, but the following endpoints were studied: thus, we report that (i) α-MSH improved postischemic cardiac function (contractility and aortic flow), (ii) reduced myocardial infarct size and the incidence of reperfusion-induced ventricular fibrillation and (iii) attenuated apoptotic cell death.

2. Materials and methods

2.1. Isolated working heart preparation

Sprague–Dawley rats (320–350 g) were anesthetized with ether then intravenous heparin (500 IU/kg) was injected. After thoracotomy, the heart was excised, the aorta was cannulated and the heart was perfused (at 37 °C) according to the Langendorff method for a 5-min washout period at a constant perfusion pressure equivalent to 100 cm of water (10 kPa). The perfusion medium consisted of a modified Krebs–Henseleit bicarbonate buffer (millimolar concentration: sodium chloride 118, potassium chloride 4.7, calcium chloride 1.7, sodium bicarbonate 25, potassium biphosphate 0.36, magnesium sulfate 1.2 and glucose 10). The Langendorff preparation was switched to the 'working' mode following the washout period as previously described by Yamamoto et al. (1984) and modified by Tosaki and Braquet (1990).

2.2. Induction of regional and global ischemia

After a 10-min aerobic perfusion of the heart, the left main coronary artery was occluded, a suture mounted on a curved needle was placed under the origin of the main descending coronary artery and the ends of the suture were passed through a small plastic tube. Myocardial ischemia was induced by clamping the plastic tube onto the surface of the heart with a surgical clamp. Reperfusion was initiated by releasing the snare. The prevention of hearts from drying out during myocardial ischemia, the thermostated glassware was covered and humidity was kept at a constant level (90%–100%). In order to avoid the high incidence of reperfusion-induced ventricular arrhythmias, hearts were initially reperfused in Langendorff mode, and potassium concentration was relatively high in the perfusion buffer (Curtis and Hearse, 1989). Infarct size was measured in hearts subjected to coronary artery occlusion (regional ischemia) in drug-free and α-MSH-treated groups. α-MSH (Sigma, Budapest, Hungary) was subcutaneously injected 12 h before the isolation of hearts and the onset of ischemia/reperfusion.

For the measurement of cardiac function (heart rate, coronary flow, aortic flow, left ventricular developed pressure), the incidence of reperfusion-induced ventricular fibrillation (VF) and DNA laddering for apoptosis, a model of global cardiac ischemia was used. Thus, after 10-min aerobic perfusion of the heart, the atrial inflow and aortic outflow lines were totally clamped at a point close to the origin of the aortic cannula. Reperfusion was initiated by unclamping the atrial inflow and aortic outflow lines. An epicardial electrocardiogram (ECG) was recorded by two silver electrodes attached directly to the heart. ECGs were analyzed to determine the incidence of ventricular fibrillation. If ventricular fibrillation was registered at the beginning of reperfusion, hearts were defibrillated and myocardial function was recorded. The heart was considered to be in ventricular fibrillation if an irregular baseline was apparent on the ECG. The heart was considered to be in sinus rhythm if normal sinus complexes occurring in a regular rhythm were apparent on the ECG. Before ischemia and during reperfusion, the values of heart rate, coronary flow and aortic flow were registered. Left ventricular developed pressure, defined as the difference between left ventricular systolic and end-diastolic pressure, was recorded by the insertion of a catheter into the left ventricle via the left atrium and mitral valve.

2.3. Determination of infarct size

Hearts for infarct size measurement were perfused, at the end of each experiment, with 30 ml of 1% triphenyl tetrazolium solution in phosphate buffer (Na_2HPO_4 88 mM, NaH_2PO_4 1.8 mM) via the side arm of the aortic cannula then stored at −70 °C for later analysis. Frozen myocardial tissue was sliced transversely (Schultz et al., 1997) in a plane perpendicular to the apico-basal axis into 1-mm thick sections, weighted, blotted dry, placed in between microscope slides and scanned on a Hewlett-Packard Scanjet 5p single pass flat bed scanner (Hewlett-Packard, Palo Alto, CA). Using the NIH Image 1.61 image processing software, each digitized image was subjected to equivalent degrees of background subtraction, brightness and contrast enhancement for improved clarity and distinctness. Risk as well as infarct zones of each slice were traced and the

respective areas were calculated in terms of pixels. At the end of each experiment, the risk area was determined by Evans blue (Mocanu et al., 2000), and the intact ventricular tissue stained in red by triphenyl tetrazolium while the infarct area (white) was unstained by triphenyl tetrazolium. The areas were measured by computerized planimetry software and these areas were multiplied by the weight of each slice, then the results summed up to obtain the weight of the risk zone (g) and the infarct zone (g). Infarct size was expressed as the ratio, in percent, of the infarct zone to the risk zone.

2.4. DNA fragmentation

Apoptosis is very well characterized biochemically by the cleavage of genomic DNA into nucleosomal fragments of 180 bp or multiples thereof that are readily detected as a DNA ladder by gel electrophoresis (Roche, Mannheim, Germany). Genomic DNA was isolated from untreated and α-MSH-treated hearts to carry out DNA laddering. Briefly, cardiac tissues (about 60 mg) were pelletted in eppendorf tubes using 1000*g* for 5 min, and supernatants were removed. Three hundred microlitres of lysis buffer [10 mM EDTA, 0.5% sarcosyl, 50 mM *tris*-hydroxymethyl)-aminomethane, pH = 8.0] were added and vortexed, then 60 μl of proteinase K (from a stock solution) were added to each sample. Mixtures were vortexed and incubated for 2 h at 55 °C, then binding buffer (300 μl) was used and samples were further incubated for 20 min at 72 °C in the presence of 150 μl of isopropanol. Gel loading buffer (5 μl) was used, and DNA samples were electrophoresed on a 1.8% agarose gel with ethidium bromide. DNA laddering was visualized and photographed under ultraviolet transillumination.

2.5. Measurement of plasma corticosterone

After 30 min and 12 h of the injection of 200 μg/kg α-MSH, blood samples were collected in heparinized glass tubes, and plasma corticosterone levels were analyzed fluorimetrically as described by Zenker and Bernstein (1958).

2.6. Statistical analysis

The values for myocardial function, infarct sizes and corticosterone levels ($n = 6$ in each group) were all expressed as the means ± standard error of the mean (S.E.M.). Two-way analysis of variance was first carried out to test for any differences between the mean values of all groups. If differences were established, the values of α-MSH-treated groups were compared with those of the drug-free control group by multiple *t*-test followed by Bonferroni correction. For the distribution of discrete variables such as the incidence of ventricular fibrillation which follows a nonparametric distribution (non-Gaussian distribution), an overall chi-square test for a $2 \times n$ table was constructed followed by a sequence of 2×2 chi-square tests to compare individual groups. For the study of the incidence of reperfusion-induced ventricular fibrillation, $n = 12$ hearts were necessary in order to make comparison between the drug-free and α-MSH-treated groups. A change of $p < 0.05$ between the drug-free control and treated groups was considered to be significant.

3. Results

Fig. 1 shows the antiapoptotic effect of α-MSH detected by agarose gel electrophoresis. Thus, DNA fragmentation was not seen under aerobic control perfusion (Fig. 1, lane 1). However, in hearts subjected to 30 min of global ischemia followed by 120 min of reperfusion, DNA laddering (Fig. 1, lane 2) was detected in the drug-free myocardium. In rats treated with 40, 200 and 400 μg/kg of α-MSH, and hearts were excised, isolated and subjected to ischemia/reperfusion, DNA fragmentation was reduced (Fig. 1, lanes 1, lanes 3, 4 and 5) indicating by the intensity of lanes. Thus, with the use of 200 and 400 μg/kg of α-MSH, the fragmentation of DNA was completely abolished in the ischemic/reperfused myocardium.

Fig. 2 shows the infarct size in drug-free controls and groups treated with various doses of (40, 200 and 400 μg/kg). Thus, with the does of 200 and 400 μg/kg of α-MSH, infarct size was significantly reduced from its control value of 38 ± 5% to 17 ± 3% and 19 ± 4%, respectively (Fig. 2). The lowest concentration of α-MSH (40 μg/kg) failed to significantly reduce the infarct size in comparison with the drug-free control value.

Table 1 shows the cardiac function obtained in rats pretreated with various doses of α-MSH, and hearts were isolated and subjected to 30 min of global ischemia fol-

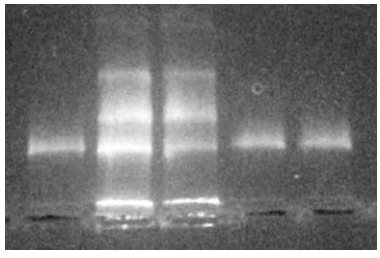

Fig. 1. DNA ladders were visible in agarose gel electrophoresis of DNA from the sample of ischemic/reperfused myocardium (lane 2). Rats were treated with 40 μg/kg (lane 3), 200 μg/kg (lane 4) and 400 μg/kg (lane 5) of α-MSH, and hearts were isolated and subjected to ischemia/reperfusion. The fragmentation of DNA was not observed in hearts of rats treated with 200 and 400 μg/kg of α-MSH. Ischemia: IS, reperfusion: RE.

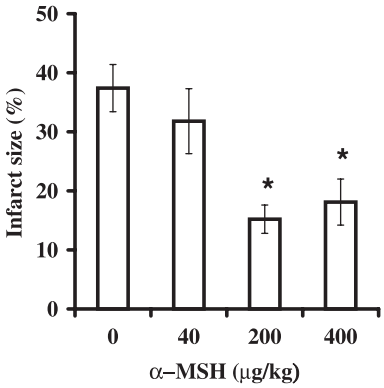

Fig. 2. The effect of α-MSH on the reduction of infarct size (%) in hearts subjected to 30 min of coronary artery ligation, followed by 2 h of reperfusion. $N=6$ in each group, means ± S.E.M., $*p<0.05$ in comparison with the drug-free control value.

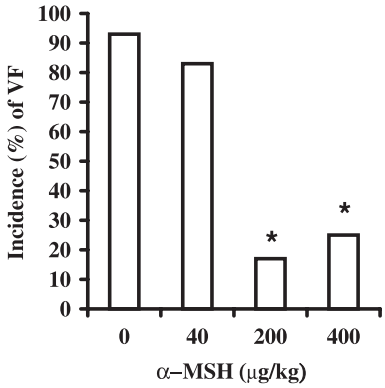

Fig. 3. The effect of various doses of α-MSH on the incidence of reperfusion-induced ventricular fibrillation in isolated ischemic/reperfused hearts. α-MSH was injected subcutaneously, and after 12 h of injection, hearts were isolated and subjected to 30-min ischemia, followed by 120 min of reperfusion. The incidence (%) of reperfusion-induced ventricular fibrillation was recorded. $N=12$ in each group, $*p<0.05$ compared to the drug-free control value.

lowed by 120 min of reperfusion. Thus, our results show that 40 μg/kg of α-MSH did not significantly change the preischemic or postischemic cardiac function (heart rate, coronary and aortic flow rates and left ventricular developed pressure). However, the increase of α-MSH dose from 40 to 200 and 400 μg/kg, a significant increase was observed in aortic flow and left ventricular developed pressure before the induction of ischemia (Table 1, preischemic values). During reperfusion (Table 1), the postischemic cardiac recovery, including coronary flow, aortic flow and left ventricular developed pressure, was significantly improved in hearts treated with 200 and 400 μg/kg of α-MSH, respectively. Interestingly, heart rate was not significantly changed either before the onset of ischemia or during reperfusion in α-MSH-treated groups in comparison with the drug-free control values.

Fig. 3 shows the incidence of reperfusion-induced ventricular fibrillation in hearts, obtained from rats treated with various doses of α-MSH, subjected to 30 min of ischemia followed by 120 min of reperfusion. It is reasonable to believe that the improvement in postischemic cardiac function (Table 1), in α-MSH-treated groups, reflected in a significant reduction in the development of reperfusion-induced ventricular fibrillation. Thus, our results show (Fig. 3) that the incidence of reperfusion-induced ventricular fibrillation was significantly reduced from its drug-free control value of 92% to 83%, 17% ($p<0.05$) and 25% ($p<0.05$) with the concentrations of 40, 200 and 400 μg/kg of α-MSH, respectively.

After 30 min and 12 h of the injection of 200 μg/kg of α-MSH, plasma corticosterone level was significantly increased from its drug-free control value of 90 ± 11 to 248 ± 16 ($p<0.05$) and 144 ± 14 μg/l ($p<0.05$). Thus, after 12 h of the injection of α-MSH, plasma corticosterone concentration (144 ± 14 μg/l) was still at a significant level in comparison with the drug-free control value (90 ± 11 μg/l), but this value was about 50% less compared to the α-MSH-induced corticosterone level at 30 min (248 ± 16 μg/l).

4. Discussion

Reperfusion of the previously ischemic myocardium has long been known to cause additional damage so called

Table 1
The effect of α-MSH on cardiac function in ischemic/reperfused hearts

Groups	Preischemic values				After 60-min RE				After 120-min RE			
	HR	CF	AF	LVDP	HR	CF	AF	LVDP	HR	CF	AF	LVDP
Control	300 ± 2	25.7 ± 0.5	48.8 ± 1.3	17.2 ± 0.5	293 ± 3	17.6 ± 0.8	23.8 ± 1.3	10.3 ± 0.5	275 ± 2	14.6 ± 0.6	7.5 ± 0.5	9.1 ± 0.4
α-MSH (40 μg)	295 ± 5	24.5 ± 0.6	51.8 ± 1.3	18.0 ± 0.6	289 ± 7	17.9 ± 0.5	24.2 ± 0.4	10.9 ± 0.3	275 ± 3	14.8 ± 0.3	8.8 ± 0.6	9.8 ± 0.3
α-MSH (200 μg)	298 ± 5	25.4 ± 0.7	60.2 ± 3.5[a]	20.9 ± 1.1[a]	291 ± 3	23.5 ± 0.7[a]	39.5 ± 1.0[a]	14.8 ± 0.6[a]	282 ± 2	20.2 ± 0.4[a]	31.5 ± 0.9[a]	15.9 ± 0.4[a]
α-MSH (400 μg)	290 ± 3	24.9 ± 0.4	63.5 ± 2.5[a]	21.7 ± 0.8[a]	290 ± 5	22.7 ± 0.8[a]	37.9 ± 0.9[a]	14.3 ± 0.4[a]	277 ± 4	20.5 ± 0.6[a]	33.5 ± 3.0[a]	16.3 ± 1.4[a]

$N=6$ in each group, means ± S.E.M. HR: heart rate (beats/min), CF: coronary flow (ml/min), AF: aortic flow (ml/min), LVDP: left ventricular developed pressure (kPa), RE: reperfusion.

[a] $p<0.05$ compared to the drug-free control group.

reperfusion-induced injury (Tennant and Wiggers, 1935). A better understanding of the factors responsible for the development of reperfusion-induced damages would be expected to facilitate assessment of their clinical relevance and therapeutic approaches of their prevention. Many factors have been suggested to play a critical role in ischemia/reperfusion-induced damage, and at present, the relative importance of these factors is uncertain and sometime controversial. However, it is reasonable to assume that a change in only one pathological factor or signal transduction mechanism, e.g., by pharmacological intervention, into the appropriate physiological direction could suppress or interfere with other pathological pathways leading to the recovery of postischemic cardiac function.

The goal of our experiments was to study whether α-MSH treatment could afford cardiac protection against ischemia/reperfusion-induced injury. Thus, we studied that (i) α-MSH is able to improve postischemic cardiac function, (ii) to reduce myocardial infarct size and the incidence of reperfusion-induced ventricular fibrillation and (iii) to attenuate apoptotic cell death. However, the primary aim of our investigation was not to study the action mechanism(s) in rats treated with α-MSH, but to clarify the outcome and final endpoints (cardiac function, infarct size, ventricular fibrillation and apoptotic cell death) of reperfusion-induced injury in the myocardium. In our study, thus, the action mechanism(s) of α-MSH remains on speculation and the possible explanation is based on previous investigations (Van Bergen et al., 1995; Versteeg et al., 1998). It is important to note that drugs, which can slow down heart rate, are able to protect the ischemic-reperfused myocardium (Tosaki et al., 1988; Bernier et al., 1989). The results of our study have shown that α-MSH inhibits reperfusion-induced damage, and the protective effect of α-MSH was unrelated to any change in heart rate. Although not conclusive in our studies, α-MSH was given before the induction of ischemia, and this would lead us to suggest that a major component of the protection of α-MSH may occur as a result of its action during the preceding period of ischemia. It is often justified on the grounds that time must be allowed for the intervention to reach the tissue and must be available to act before the critical early moments of ischemia and reperfusion. Thus, acceptable evidence for ischemia/reperfusion injury could only be obtained from additional studies in which α-MSH is given at the onset of reperfusion. This conclusion could be supported by the fact that in hearts obtained from rats treated with 200 or 400 μg/kg of α-MSH, the preischemic values of aortic flow and left ventricular developed pressure were significantly increased in comparison with the corresponding drug-free control values.

Apoptosis frequently occurs in both chronic and acute tissue injury and the signal and molecular mechanisms responsible for this form of cell death are not precisely known. In our present study, we did not investigate whether free radicals (Von Harsdorf et al., 1999), tumor necrosis factor-α (Kurrelmeyer et al., 2000), protein kinase (Gottlieb et al., 1994), caspases (De Moissac et al., 2000; Mocanu et al., 2000), p53 (Bialik et al., 1997; Leri et al., 1998), p38 mitogen-activated protein kinase (Bogoyevitch et al., 1996; Ma et al., 1999) and calcium (James et al., 1996; Valente et al., 1998) signal mechanism mediate apoptotic cell death, although these mediators have been suggested and frequently cited as important culprits. The data, as one of the endpoints of our study, show that α-MSH at concentrations of 200 and 400 μ/kg reduced the apoptotic cell death. One of the possible explanations of α-MSH- induced antiapoptotic mechanism could be based on the finding of Jo et al. (2001), who emphasized that α-MSH significantly reduced Fas and Fas ligand protein expression leading to the protection of ischemic/reperfused tissue. α-MSH-induced protection against apoptotic cell death was reported by Jo et al. (2001), and the same protection was detected by another active fragment of ACTH, a melanocortin peptide, in the studies of Bazzani et al. (2001).

It is reasonable to assume that a reduction in apoptotic cell death limits infarct size in α-MSH-treated subjects because apoptotic cell death was detected, beside the necrotic cells, in the infarcted area (Maulik et al., 1998; Elsasser et al., 2000; Jonassen et al., 2000). However, it is not clear and is not the aim of our present study to what extent of apoptosis and necrosis individually contribute to the development of myocardial infarction, and probably both of them, a ‘‘necro-apoptotic’’ mechanism contributes to the development of reperfusion-induced injury and cell death. On the other hand, α-MSH and melanocortin peptides have been shown to inhibit inflammation under many experimental conditions, such as arthritis, inflammation and ischemia (Wikberg, 1999). The antiinflammatory effects of α-MSH and melanocortin peptides are often associated with a reduced production of proinflammatory cytokines, such as interleukin-1α, interleukin-1β, interleukin-6 and tumor necrosis factor-α (Luger et al., 1997; Lipton et al., 1998), and with an enhanced genesis of the antiinflammatory interleukin-10 and of the angiogenic factor interleukin-8 (Luger et al., 1997). Thus, it is reasonable to believe that endogenous antiinflammatory mediators such as α-MSH could contribute to the reduced release of inflammatory mediators in the heart and other organs (Airaghi et al., 1995, 2000) in humans. Furthermore, it is also possible that other action mechanisms of α-MSH and melanocortin peptides could be involved in the cardioprotective activity, as is the case for various cardiovascular effects of ACTH fragments, particularly under conditions of circulatory shock (Bertolini, 1995; Guarini et al., 1999) and hypoxia/reoxygenation induced by prolonged respiratory arrest (Guarini et al., 1997).

One of the objectives of our study was to ascertain whether the antifibrillatory effect observed during reperfusion was a direct consequence of the actions of α-MSH during the reperfusion period or whether they were secondary to some effects operative during ischemia. The drug was subcutaneously injected to rats 12 h prior to the

isolation of hearts and the induction of ischemia/reperfusion, therefore, this would lead us to support that antifibrillatory effect of α-MSH during reperfusion may occur, at least in part, as a result of its action during the preceding period of cardiac ischemia. It is of interest to note the finding of Vecsernyes et al. (2000), indicating that α-MSH pretreatment could increase the concentration of corticosterones in blood and, as a consequence, corticosterones could protect the ischemic/reperfused tissues (Valen et al., 2000a, b; Pearl et al., 2002) probably via heat shock protein 72 and free radical mechanisms. The fact that free radical mediated mechanism is involved in melanocortin peptide-induced cardiac protection is elegantly proven in the electron spin resonance study of Bazzani et al., (2001). The antifibrillatory effect of α-MSH would be precisely approached in an additional study designed with the drug administration at the moment of reperfusion. However, in the studies of Guarini et al., (2002), it was suggested that melanocortins could protect the myocardium and reduce the incidence of arrhythmias via the stimulation of melanocortin receptor-3 (Guarini et al., 2002).

In conclusion, the finding of the present study demonstrates that melanocortin peptides, i.e., α-MSH, significantly attenuate the consequences of myocardial ischemia/reperfusion. However, there is a clear need for further extensive and careful investigation in the field of ischemia/reperfusion-induced injury in order to precisely clarify the action mechanism(s) of α-MSH, an active fragment of ACTH, including arrhythmias, apoptotic and necrotic cell death and cardiac function. Furthermore, these results are rather exciting, because they could disclose a new therapeutic approach in the treatment of ischemia/reperfusion-induced injury.

Acknowledgements

This study was supported by the grants from the ETT (18/2001, 62/2001) and OTKA (T-32008), Hungary. Dr M. Vecsernyes was supported by the Szechenyi Foundation, Hungary.

References

Airaghi, L., Lettino, M., Manfredi, M.G., Lipton, J.M., Catania, A., 1995. Endogenous cytokine antagonists during myocardial ischemia and thrombolytic therapy. Am. Heart J. 130, 204–211.

Airaghi, L., Garofalo, L., Grazia-Cutuli, M., Delgado, R., Carlin, A., Demitri, M.T., Badalamenti, S., Graziani, G., Lipton, J.M., Catania, A., 2000. Plasma concentrations of α-melanocyte-stimulating hormone are elevated in patients on chronic haemodialysis. Nephrol. Dial. Transplant. 15, 1212–1216.

Avkiran, M., Gross, G., Karmazyn, M., Klein, H., Murphy, E., Ytrehus, K., 2001. Na^+/H^+ exchange in ischemia, reperfusion and preconditioning. Cardiovasc. Res. 50, 162–166.

Bazzani, C., Guarini, S., Botticelli, A.R., Zaffe, D., Tomasi, A., Bini, A., Cainazzo, M.M., Ferrazza, G., Mioni, C., Bertolini, A., 2001. Protective effect of melanocortin peptides in rat myocardial ischemia. J. Pharmacol. Exp. Ther. 297, 1082–1087.

Bernier, M., Curtis, M.J., Hearse, D.J., 1989. Ischemia-induced and reperfusion-induced arrhythmias: importance of heart rate. Am. J. Physiol. 256, H21–H31.

Bertolini, A., 1995. The opioid/antiopioid balance in shock: a new target for therapy in resuscitation. Resuscitation 30, 29–42.

Bertolini, A., Guarini, S., Rompianesi, E., Ferrari, W., 1986. α-MSH and other ACTH fragments improve cardiovascular function and survival in experimental hemorrhagic shock. Eur. J. Pharmacol. 130, 19–26.

Bertolini, A., Guarini, S., Ferrari, W., Noera, G., Massini, C., Di Tizio, S., 1987. ACTH-(1–24) restores blood pressure in acute hypovolaemia and haemorrhagic shock in humans. Eur. J. Clin. Pharmacol. 32, 537–538.

Bialik, S., Geenen, D.L., Sasson, I.E., Cheng, R., Horner, J.W., Evans, S.M., Lord, E.M., Koch, C.J., Kitsis, R.N., 1997. Myocyte apoptosis during acute myocardial infarction in the mouse localizes to hypoxic regions but occurs independently of p53. J. Clin. Invest. 100, 1363–1372.

Bogoyevitch, M.A., Gillespie-Brown, J., Ketterman, A.J., Fuller, S.J., Ben-Levy, R., Ashworth, A., Marshall, C.J., Sugden, P.H., 1996. Stimulation of the stress-activated mitogen-activated protein kinase subfamilies in perfused heart: p38/RK mitogen activated protein kinases and c-Jun N-terminal kinases are activated by ischemia/reperfusion. Circ. Res. 79, 162–173.

Bolli, R., 1988. Oxygen-derived free radicals and postischemic myocardial dysfunction ("stunned myocardium"). Am. J. Coll. Cardiol. 12, 239–249.

Chiao, H., Kohda, Y., McLeroy, P., Craig, L., Housini, I., Star, R.A., 1997. α-Melanocyte-stimulating hormone protects against renal injury after ischemia in mice and rats. J. Clin. Invest. 99, 1165–1172.

Curtis, M.J., Hearse, D.J., 1989. Ischemia-induced and reperfusion-induced arrhythmias differ in their sensitivity to potassium. J. Mol. Cell. Cardiol. 21, 21–40.

Curtis, M.J., Pugsley, M.K., Walker, M.J.A., 1993. Endogenous chemical mediators of ventricular arrhythmias in ischemic heart disease. Cardiovasc. Res. 27, 703–719.

De Groot, M.J., Coumans, W.A., Willemsen, P.H., van der Vusse, G.J., 1993. Substrate-induced changes in the lipid content of ischemic and reperfused myocardium. Its relation to hemodynamic recovery. Circ. Res. 72, 176–186.

De Moissac, D., Gurevich, R.M., Zheng, H., Singal, P.K., Kirshenbaum, L.A., 2000. Caspase activation and mitochondrial cytochrome *c* release during hypoxia-mediated apoptosis of adult ventricular myocytes. J. Mol. Cell. Cardiol. 32, 53–63.

Elsasser, A., Suzuki, K., Schaper, J., 2000. Unresolved issues regarding the role of apoptosis in the pathogenesis of ischemic injury and heart failure. J. Mol. Cell. Cardiol. 32, 711–724.

Gottlieb, R.A., Burleson, K.O., Kloner, R.A., Babior, B.M., Engler, R.L., 1994. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. J. Clin. Invest. 94, 1621–1628.

Guarini, S., Bazzani, C., Bertolini, A., 1997. Resuscitating effect of melanocortin peptides after prolonged respiratory arrest. Br. J. Pharmacol. 121, 1454–1460.

Guarini, S., Bazzani, C., Cainazzo, M.M., Mioni, C., Ferrazza, G., Vergoni, A.V., Schioth, H.B., Wikberg, J.E.S., Bertolini, A., 1999. Evidence that melanocortin 4 receptor mediates hemorrhagic shock reversal caused by melanocortin peptides. J. Pharmacol. Exp. Ther. 291, 1023–1027.

Guarini, S., Schioth, H.B., Mioni, C., Cainazzo, M.M., Ferrazza, G., Giuliani, D., Wikberg, J.E.S., Bertolini, A., Bazzani, C., 2002. MC_3 receptors are involved in the protective effect of melanocortins in myocardial ischemia/reperfusion-induced arrhythmias. Naunyn-Schmiedeberg's Arch. Pharmacol. 366, 177–182.

Hearse, D.J., Bolli, R., 1992. Reperfusion-induced injury: manifestations, mechanisms, and clinical relevance. Cardiovasc. Res. 26, 101–108.

Hearse, D.J., Humphrey, S.M., Bullock, G.R., 1978. The oxygen paradox and the calcium paradox: two facets of the same problem? J. Mol. Cell. Cardiol. 10, 641–668.

James, T.N., St. Martin, E., Willis, P.W., Lohr, T.O., 1996. Apoptosis as a possible cause of gradual development of complete heart block and fatal arrhythmias associated with absence of the AV node, sinus node, and internodal pathways. Circulation 93, 1424–1438.

Jo, S.K., Yun, S.Y., Chang, K.H., Cha, D.R., Cho, W.Y., Kim, H.K., Won, N.H., 2001. α-MSH decreases apoptosis in ischemic acute renal failure in rats: possible mechanism of this beneficial effect. Nephrol. Dial. Transplant. 16, 1583–1591.

Jonassen, A.K., Brar, B.K., Mjos, O.D., Sack, M.N., Latchman, D.S., Yellon, D.M., 2000. Insulin administered at reoxygenation exerts a cardioprotective effect in myocytes by a possible anti-apoptotic mechanism. J. Mol. Cell. Cardiol. 32, 757–764.

Keating, M.T., Sanguinetti, M.C., 2001. Molecular and cellular mechanisms of cardiac arrhythmias. Cell 104, 569–580.

Kolbeck-Ruhmkorff, C., Zimmer, H.G., 1995. Proto-oncogene expression in the isolated working rat heart: combination of pressure and volume overload with norepinephrine. J. Mol. Cell. Cardiol. 27, 501–511.

Kurrelmeyer, K.A., Michael, L.H., Baumgarten, G., Taffet, G.E., Peschon, J.J., Sivasubramanian, N., Entman, M.L., Mann, D.L., 2000. Endogenous tumor necrosis factor protects the adult cardiac myocyte against ischemic-induced apoptosis in a murine model of acute myocardial infarction. Proc. Natl. Acad. Sci. U. S. A. 97, 5456–5461.

Leri, A., Claudio, P.P., Li, Q., Wang, X., Reiss, K., Wang, S., Malhotra, A., Kajstura, J., Anversa, 1998. Stretch-mediated release of angiotensin II induces myocyte apoptosis by activating p53 that enhances the local rennin–angiotensin system and decreases the Bcl-2-to-Bax protein ratio in the cell. J. Clin. Invest. 101, 1326–1342.

Lipton, J.M., Catania, A., Delgado, R., 1998. Peptide modulation of inflammatory processes within the brain. Neuroimmunomodulation 5, 178–183.

Luger, T.A., Scholzen, T., Grabbe, S., 1997. The role of α-melanocyte-stimulating hormone in cutaneous biology. J. Investig. Dermatol. Symp. Proc. 2, 87–93.

Ma, X.L., Kumar, S., Gao, F., Louden, C.S., Lopez, B.L., Christopher, T.A., Wang, C., Lee, J.C., Feuerstein, G.Z., Yue, T.L., 1999. Inhibition of p38 mitogen-activated protein kinase decreases cardiomyocyte apoptosis and improves cardiac function after myocardial ischemia and reperfusion. Circulation 99, 1685–1691.

Maulik, N., Kagan, V.E., Tyurin, V.A., Das, D.K., 1998. Redistribution of phosphatidylethanolamine and phosphatidylserine precedes reperfusion-induced apoptosis. Am. J. Physiol. 274, H242–H248.

Mocanu, M.M., Baxter, G.F., Yellon, D.M., 2000. Caspase inhibition and limitation of myocardial infarct size: protection against lethal reperfusion injury. Br. J. Pharmacol. 130, 197–200.

Noera, G., Angiello, N., Biagi, B., Pensa, P., 1991. Haemorrhagic shock in cardiac surgery. Pharmacological treatment with ACTH-(1–24). Resuscitation 22, 123–127.

Opie, L.H., 1991. Role of calcium and other ions in reperfusion injury. Cardiovasc. Drugs Ther. 5, 237–248.

Pearl, J.M., Nelson, D.P., Schwartz, S.M., Wagner, C.J., Bauer, S.M., Setser, E.A., Duffy, J.Y., 2002. Glucocorticoids reduce ischemia–reperfusion-induced myocardial apoptosis in immature hearts. Ann. Thorac. Surg. 74, 830–836 Discussion 836–837.

Schultz, J.E.J., Yao, Z., Cavero, I., Gross, G.J., 1997. Glibenclamide-induced blockade of ischemic preconditioning is time dependent in intact rat heart. Am. J. Physiol. 272, H2607–H2615.

Sheridan, D.J., Penkoske, P.A., Sobel, B.E., Corr, P.B., 1980. Alpha adrenergic contributions to dysrhythmias during myocardial ischemia and reperfusion. J. Clin. Invest. 65, 161–171.

Simkovich, B.Z., Abdishoo, S., Poizat, C., Hale, S.L., Kedes, L.H., Kloner, R.A., 2002. Gene activity changes in ischemically preconditioned rabbit heart gene: discovery array study. Heart Dis. 4, 63–69.

Squadrito, F., Guarini, S., Altavilla, D., Squadrito, G., Campo, G.M., Arlotta, M., Quartarone, C., Saitta, A., Cucinotta, D., Bazzani, C., Cainazzo, M.M., Mioni, C., Bertolini, A., Caputi, A.P., 1999. Adrenocorticotropin reverses vascular dysfunction and protects against splanchnic artery occlusion shock. Br. J. Pharmacol. 128, 816–822.

Suzuki, K., Murtuza, B., Sammut, I.A., Latif, N., Jayakumar, J., Smolenski, R.T., Kaneda, Y., Sawa, Y., Matsuda, H., Yacoub, M.H., 2002. Heat shock protein 72 enhances manganese superoxide dismutase activity during myocardial ischemia–reperfusion injury, associated with mitochondrial protection and apoptosis reduction. Circulation 106 (12 Suppl. 1), I270–I276.

Tennant, R., Wiggers, C.J., 1935. The effects of coronary occlusion on myocardial contraction. Am. J. Physiol. 112, 351–361.

Tosaki, A., Braquet, P., 1990. DMPO and reperfusion injury: arrhythmia, heart function, electron spin resonance, and nuclear magnetic resonance studies in isolated guinea pig hearts. Am. Heart J. 120, 819–830.

Tosaki, A., Balint, S., Szekeres, L., 1988. Pacing and reperfusion-induced arrhythmias: protection by slow heart rate in the rat heart. Cardiovasc. Res. 22, 818–825.

Valen, G., Kawakami, T., Tahepold, P., Dumitrescu, A., Lowbeer, C., Vaage, J., 2000a. Glucocorticoid pretreatment protects cardiac function and induces cardiac heat shock protein 72. Am. J. Physiol., Heart Circ. Physiol. 279, H836–H843.

Valen, G., Kawakami, T., Tahepold, P., Starkopf, J., Kairane, C., Dumitrescu, A., Lowbeer, C., Zilmer, M., Vaage, J., 2000b. Pretreatment with methylprednisolone protects the isolated rat heart against ischaemic and oxidative damage. Free Radic. Res. 33, 31–43.

Valente, M., Calabrese, F., Thiene, G., 1998. In vivo evidence of apoptosis in arrhythmogenic right ventricular cardiomyopathy. Am. J. Pathol. 152, 479–484.

Van Bergen, P., Janssen, P.M.L., Hoogerhout, P., De Wildt, D.J., Versteeg, D.H.G., 1995. Cardiovascular effects of ã-MSH/ACTH-like peptides: structure–activity relationship. Eur. J. Pharmacol. 294, 795–803.

Van der Vusse, G.J., Van Bilsen, M., Reneman, R.S., 1994. Ischemia and reperfusion-induced alterations in membrane phospholipids: an overview. Ann. N.Y. Acad. Sci. 723, 1–14.

Vecsernyes, M., Biro, E., Gardi, J., Julesz, J., Telegdy, G., 2000. Involvement of endogenous corticotropin-releasing factor in mediation of neuroendocrine and behavioral effects to alpha-melanocyte-stimulating hormone. Endocr. Res. 26, 347–356.

Versteeg, D.H.G., Van Bergen, P., Adan, R.A.H., De Wildt, A.D.J., 1998. Melanocortins and cardiovascular regulation. Eur. J. Pharmacol. 360, 1–14.

Von Harsdorf, R., Li, P.F., Dietz, R., 1999. Signalling pathways in reactive oxygen species-induced cardiomyocytes apoptosis. Circulation 99, 2934–2941.

Wikberg, J.E.S., 1999. Melanocortin receptors: perspectives for novel drugs. Eur. J. Pharmacol. 375, 295–310.

Yamamoto, F., Braimbridgem, M.V., Hearse, D.J., 1984. Calcium and cardioplegia. The optimal calcium content for the St. Thomas' Hospital cardioplegic solution. J. Thorac. Cardiovasc. Surg. 87, 908–912.

Zenker, N., Bernstein, D.E., 1958. The estimation of small amounts of corticosterone in rat plasma. J. Biol. Chem. 231, 695–701.