European Journal of Pharmacology 745 (2014) 108–116

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular pharmacology

Protective effects of the melanocortin analog NDP-α-MSH in rats undergoing cardiac arrest

Alessandra Ottani [a], Laura Neri [a], Fabrizio Canalini [a], Anita Calevro [a], Rosario Rossi [b], Gianni Cappelli [c], Marco Ballestri [c], Daniela Giuliani [a,*], Salvatore Guarini [a,*]

[a] Department of Biomedical, Metabolic and Neural Sciences, Section of Pharmacology and Molecular Medicine, University of Modena and Reggio Emilia, Modena, Italy
[b] Division of Cardiology, University of Modena and Reggio Emilia, Modena, Italy
[c] Division of Nephrology, University of Modena and Reggio Emilia, Modena, Italy

ARTICLE INFO

Article history:
Received 22 September 2014
Received in revised form
9 October 2014
Accepted 10 October 2014
Available online 22 October 2014

Keywords:
Cardiac arrest
Metabolic acidosis
Epinephrine
Melanocortins
Resuscitation
JAK/ERK/STAT signaling

ABSTRACT

We previously reported that melanocortins afford cardioprotection in conditions of experimental myocardial ischemia/reperfusion, with involvement of the janus kinases (JAK), extracellular signal-regulated kinases (ERK) and signal transducers and activators of transcription (STAT) signalings. We investigated the influence of the melanocortin analog [Nle^4, D-Phe^7]α-melanocyte-stimulating hormone (NDP-α-MSH) on short-term detrimental responses to cardiac arrest (CA) induced in rats by intravenous (i.v.) administration of potassium chloride, followed by cardiopulmonary resuscitation (CPR) plus epinephrine treatment. In CA/CPR rats i.v. treated with epinephrine (0.1 mg/kg) and returned to spontaneous circulation (48%) we recorded low values of mean arterial pressure (MAP) and heart rate (HR), alteration of hemogasanalysis parameters, left ventricle low expression of the cardioprotective transcription factors pJAK2 and pTyr-STAT3 (JAK-dependent), increased oxidative stress, up-regulation of the inflammatory mediators tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), and down-regulation of the anti-inflammatory cytokine IL-10, as assessed at 1 h and 3 h after CPR. On the other hand, i.v. treatment during CPR with epinephrine plus NDP-α-MSH (340 μg/kg) almost completely restored the basal conditions of MAP and HR, reversed metabolic acidosis, induced left ventricle up-regulation of pJAK2, pTyr-STAT3 and IL-10, attenuated oxidative stress, down-regulated TNF-α and IL-6 levels, and improved survival rate by 81%. CA/CPR plus epinephrine alone or in combination with NDP-α-MSH did not affect left ventricle pSer-STAT3 (ERK1/2-dependent) and pERK1/2 levels. These results indicate that melanocortins improve return to spontaneous circulation, reverse metabolic acidosis, and inhibit heart oxidative stress and inflammatory cascade triggered by CA/CPR, likely via activation of the JAK/STAT signaling pathway.

1. Introduction

Cardiac arrest (CA) remains a major health problem worldwide and, although advances in cardiopulmonary resuscitation (CPR) are able to improve return to spontaneous circulation, only less than 30% of patients are discharged alive from hospital (Nichol et al., 2008; Parnia et al., 2014; Rea et al., 2004; Thom et al., 2006). Generally, these patients die for brain and cardiovascular damage, as a consequence of global ischemia/reperfusion injuries, and a high percentage of survivors have moderate to severe cognitive deficits in the months following resuscitation (Chang et al., 2007; Peberdy et al., 2010). Epinephrine is routinely used during chest compression to improve return to spontaneous circulation, being able to augment both aortic diastolic and coronary perfusion pressures (Lin et al., 2014; Ornato, 2008; Paradis et al., 1991). However, epinephrine exacerbates myocardial ischemia/reperfusion injury and post-resuscitation heart dysfunction by increasing myocardial oxygen demand, and it fails to improve survival and neurological outcome to hospital discharge (Lin et al., 2014; Yu et al., 2013).

In CA patients, brain and heart damage occurring during ischemia and reperfusion are due to free radical discharge,

Abbreviations: ACTH-(1-24), adrenocorticotropic hormone (1-24); CA, Cardiac arrest; CPR, cardiopulmonary resuscitation; ECG, electrocardiogram; ERK, extracellular signal-regulated kinases; HCO_3^-, bicarbonate; HR, heart rate; Htc, hematocrit; IL-6, interleukin-6; IL-10, interleukin-10; JAK, janus kinases; MAP, mean arterial pressure; MDA, malondialdehyde; NDP-α-MSH, [Nle4, D-Phe7]α-melanocyte-stimulating hormone; PCO_2^-, carbon dioxide partial pressure; PO_2, oxygen partial pressure; SBE, standard base excess; SO_2, oxygen saturation; STAT, signal transducers and activators of transcription; TNF-α, tumor necrosis factor-α

* Corresponding authors. Tel.: +39 059 2055371; fax: +39 059 2055376.
E-mail addresses: daniela.giuliani@unimore.it (D. Giuliani), salvatore.guarini@unimore.it (S. Guarini).

http://dx.doi.org/10.1016/j.ejphar.2014.10.022
0014-2999/

inflammatory and apoptotic responses (Chalkias and Xanthos, 2012; Lee et al., 2012; Meybohm et al., 2009; Norman et al., 2011). Besides further studies on therapeutic hypothermia – mainly known as efficient treatment for neuroprotection during CA – novel approaches to protect the heart and brain are under investigation, including hydrogen sulfide donors, activators of cholinergic pathways, carvedilol, and argon (Brücken et al., 2014; Kida et al., 2012; Kurita et al., 2010; Lee et al., 2012; Norman et al., 2011; Parnia et al., 2014; Yu et al., 2013).

The endogenous peptides melanocortins, namely adrenocorticotropin/melanocyte-stimulating hormone (ACTH/MSH) group [ACTH-(1-24), α-MSH, and shorter fragments] (Catania et al., 2004; Corander et al., 2009; Giuliani et al., 2012; Wikberg and Mutulis, 2008), as well as synthetic analogs, exert cardioprotective effects. These neuropeptides have been shown to attenuate free radical discharge, inflammatory and apoptotic responses induced by myocardial ischemia/reperfusion in rats and mice, to stimulate anti-apoptotic reactions, and to reduce reperfusion-induced arrhythmias as well as infarct size (Bazzani et al., 2002; Getting et al., 2006; Mioni et al., 2003, 2005; Ottani et al., 2010). Further, melanocortins have a resuscitating effect during respiratory arrest and hemorrhagic shock, and afford neuroprotection in conditions of global and focal brain ischemia (Bertolini et al., 1989; Giuliani et al., 2007b, 2012; Guarini et al., 1997a). Recently we have shown that janus kinases (JAK), extracellular signal-regulated kinases (ERK) and signal transducers and activators of transcription (STAT) – signaling pathways that play an established cardioprotective role (Bolli et al., 2011; Minamino, 2012; Obana et al., 2012; Xuan et al., 2001, 2005) – are involved in the cardioprotective effect of melanocortins against ischemia/reperfusion-induced damage (Ottani et al., 2013).

Overall, these findings led us to hypothesize that melanocortins could improve resuscitation and/or induce cardioprotection also in conditions of CA. Here we investigated, therefore, the effect of the melanocortin analog [Nle^4, D-Phe^7]α-MSH (NDP-α-MSH) in combination with epinephrine in a rat model of CA followed by CPR, focusing our study on resuscitation success, metabolic acidosis, JAK/ERK/STAT signaling, oxidative stress, inflammatory and apoptotic responses.

2. Materials and methods

2.1. Animals, surgery and cardiac arrest induction

Adult male Sprague–Dawley rats (Charles River, Milan, Italy), weighing 400–450 g, were used. They were kept in air conditioned colony rooms (temperature 21 ± 1 °C; humidity 60%) on a natural light/dark cycle, with food in pellets and tap water available ad libitum. Housing conditions and experimental procedures were in strict accordance with the European Community regulations on the use and care of animals for scientific purposes (CEE Council 89/609; Italian D.L. 22-1-92 No. 116), and were approved by the Animal Ethics Committee of Modena and Reggio Emilia University.

Under general anesthesia (urethane, 1.25 g/kg intraperitoneally) and after heparinization (heparin sodium, 600 iu/kg intravenously; i.v.), rats were fixed in the supine position on a heated operating platform, so to maintain rectal temperature close to 37 °C; this procedure was adopted to avoid wrong interpretation of data due to a possible protective role of hypothermia (Lee et al., 2012; Norman et al., 2011). Rats were instrumented with indwelling polyethylene catheters in a common carotid artery, to record arterial blood pressure, and into an iliac vein for treatments and blood sampling. The arterial catheter was connected to a pressure transducer coupled to a Surveyor apparatus (Mortara-Rangoni, Bologna, Italy), as previously described (Guarini et al., 1996, 1997a, 1997b, 2003). After cannulation of the trachea the animals were ventilated with room air by means of a respirator for small rodents with a stroke volume of approximately 20 ml/kg and a rate of 70 strokes/min; these ventilation parameters maintained arterial pO_2, pCO_2 and pH close to the normal range (Mioni et al., 2003, 2005; Ottani et al., 2010, 2013). The lead II electrocardiogram (ECG) was recorded by means of needle electrodes placed subcutaneously on the limbs and connected to the Surveyor apparatus, as previously described (Mioni et al., 2003, 2005; Ottani et al., 2010, 2013). Arterial blood pressure and ECG were continuously monitored in all animals until kill (3 h after return to spontaneous circulation).

After a 10 min equilibration period, CA was induced by i.v. administration of 70 mg/kg potassium chloride (KCl) dissolved in saline (1 ml/kg) and mechanical ventilator turning off, and was confirmed by loss of arterial pressure and asystolic rhythm on ECG (Angelos et al., 2011; Kida et al., 2012; Norman et al., 2011). After a 5 min arrest time, chest compressions were delivered by using two fingers at a rate of 200–220 beats/min until resuscitation (and for a maximum of 7 min) followed by resumption of mechanical ventilation; rats not resuscitated within 7 min were discarded. One minute after starting CPR, epinephrine alone or epinephrine plus NDP-α-MSH were i.v. bolus injected, as detailed in Section 2.2. Also animals resuscitated but not surviving at the 3 h observation period were discarded. Sham CA/CPR rats were subjected to all surgical procedures experienced by CA/CPR animals, but saline instead of KCl was administered.

2.2. Drugs, treatments and experimental groups

NDP-α-MSH, synthetic melanocortin analog with long-lasting biological activity (Giuliani et al., 2006, 2007a, 2007b, 2009; Ottani et al., 2010, 2013) (kindly provided by Prof. Paolo Grieco, University of Naples Federico II), and epinephrine (S.A.L.F., Cenate Sotto, Italy) were used. Epinephrine was administered at the dose of 0.1 mg/kg (Angelos et al., 2011; Yu et al., 2013), whereas the dose of NDP-α-MSH (340 μg/kg) was chosen on the basis of previous experiments performed in our laboratory in rats subjected to myocardial ischemia/reperfusion (Ottani et al., 2010, 2013). Both drugs were freshly dissolved in saline immediately before use and injections were performed i.v., in a volume of 1 ml/kg, 1 min after starting CPR (epinephrine followed by the immediate delivery of NDP-α-MSH). Animals were randomly assigned to the following experimental groups: 1) sham CA/CPR + saline; 2) CA/CPR + saline; 3) CA/CPR + epinephrine; 4) CA/CPR + epinephrine + NDP-α-MSH; 5) sham CA/CPR + epinephrine + NDP-α-MSH. Because of the very low survival rate (14%) in this experimental model of CA/CPR, the experimental control group of CA/CPR rats treated with saline was only used for evaluating survival.

2.3. Hemogasanalysis and other blood measurements

Measurements were performed in rats of all experimental groups. Blood samples (0.3 ml) were taken from the venous catheter just before CA (basal conditions), 1 and 3 h after CPR, and analyzed for pH, oxygen partial pressure (PO_2), carbon dioxide partial pressure (PCO_2), bicarbonate (HCO_3^-), standard base excess (SBE), oxygen saturation (SO_2), lactate, hematocrit (Hct), Na^+, Ca^{2+} and K^+ by using an ABL90 FLEX analyzer (Radiometer Medical ApS, Brønshøj, Denmark).

2.4. Assay of malondialdehyde concentration

Oxidative stress was assessed in the left ventricle by measuring concentration of malondialdehyde (MDA; marker of lipid peroxidation index) using a colorimetric commercial kit (Lipid peroxidation assay

kit, Calbiochem, San Diego, CA) (Giuliani et al., 2014). Briefly, at the end of the 3 h period of return to spontaneous circulation, in rats of each group the left ventricle was removed under deep anesthesia. In 6 rats per group, left ventricle was blotted dry and then weighed. After centrifugation of the left ventricle lysate, 0.65 ml of 10.3 mM *N*-methyl-2phenyl-indole in acetonitrile were added to 0.2 ml of the supernatant. After vortexing for 3–4 s and adding 0.15 ml of HCl 37%, samples were mixed well and closed with a tight stopper and incubated at 45 °C for 60 min. The samples were then cooled on ice and optical density was spectrophotometrically measured at 532 nm (340 ATTC; SLT Lab Instruments, Grödig, Austria). MDA concentration was calculated from a standard curve established with serial dilutions of tetraethoxypropane, and expressed as nmol/g of tissue.

2.5. Isolation of cytoplasmatic proteins and Western blot analysis

At the end of the 3 h period of return to spontaneous circulation, in the remaining rats (7–10 per group) the left ventricle was used for Western blot analysis. Isolation of cytoplasmatic proteins and Western blot analysis were performed as previously described (Giuliani et al., 2006; Ottani et al., 2010, 2013). Briefly, after homogenization in lysis buffer and centrifugation at 15,000*g* for 15 min, the supernatant was collected and employed for protein determination using the BioRad Protein Assay Kit (BioRad, Richmond, USA). Cytoplasmatic proteins were denatured in reducing buffer and separated by electrophoresis, and the separated proteins (40 μg for each sample) were transferred onto polyvinylidene fluoride membranes using a transfer buffer. Staining of the blots with Ponceau's solution showed that total protein amount was equal in each lane. The membranes were then blocked, washed and incubated with a primary antibody for phosphorylated (p: active form) pJAK2, pERK1/2, pTyr-STAT3 and pSer-STAT3, total JAK, total ERK1/2, total STAT3, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), BAX and active caspase-3 (Cell Signaling, Charlottesville, VA), and IL-10 (Epitomics, Burlingame, CA), overnight at 4 °C. The day after, the membranes were washed three times and incubated with a specific secondary antibody peroxidase-conjugated (Cell Signaling) for 1 h at room temperature. To prove equal loading, the blots were analyzed for β-tubulin expression (house-keeping gene) using an anti-β-tubulin antibody (Cell Signaling). The membranes were analyzed by the enhanced chemiluminescence's system according to the manufacturer's protocol (Millipore, Billerica, MA). Protein signals were quantified by scanning densitometry using a bio-image analysis system (Bio-Profil, Celbio, Italy) (Ottani et al., 2010, 2013). The level/activity of all markers was expressed as relative integrated intensity normalized versus β-tubulin (=100). The JAK, ERK1/2, Tyr-STAT3 and Ser-STAT3 signals are shown as the ratio of the integrated intensity of phosphorylated vs unphosphorylated form.

2.6. Statistical analysis

All data were collected and analyzed blind to the treatment. Survival rates were compared by Fisher's exact probability test. Mean arterial pressure (MAP) and heart rate (HR) values, and hemogasanalysis/blood parameters, were analyzed by means of two-way repeated measures ANOVA followed by Student–Newman–Keuls' test. One-way ANOVA followed by Student–Newman–Keuls' test was used for all other data. A P value <0.05 was considered significant.

3. Results

3.1. NDP-α-MSH improves return to spontaneous circulation

In all experimental groups, a part of rats undergone CA returned to spontaneous circulation within 2–4 min of chest compression, whereas a part died for CPR failure or within 30–60 min after CPR. Specifically, at the end of the 3 h period of return to spontaneous circulation, CPR was associated with a 14% survival in saline-treated rats, statistically different from that observed in epinephrine-treated animals (48%) (Table 1). Furthermore, survival rate increased significantly to 81% when NDP-α-MSH was associated with epinephrine (Table 1). Treatment of sham CA/CPR animals with i.v. epinephrine plus NDP-α-MSH did not affect survival (100%).

Table 1
NPD-α-MSH improves survival in rats subjected to CA/CPR and treated with epinephrine.

Experimental group	Treatment	Survival rate at 3 h after CPR
Sham	Saline	15/15 (100%)[a]
CA/CPR	Saline	2/14 (14%)
CA/CPR	Epinephrine	16/33 (48%)[b]
CA/CPR	Epinephrine + NDP-α-MSH	13/16 (81%)[a,c]
Sham	Epinephrine + NDP-α-MSH	9/9 (100%)[a]

CA=Cardiac arrest; CPR=Cardiopulmonary resuscitation; Sham=sham CA/CPR; Epinephrine (0.1 mg/kg, i.v.); NDP-α-MSH (340 μg/kg, i.v.).

[a] $P<0.001$ vs CA/CPR+saline.
[b] $P<0.05$ vs CA/CPR+saline.
[c] $P<0.05$ vs CA/CPR+epinephrine (Fisher's test).

During CA, in all rats there was a loss of arterial pressure and asystolic rhythm on ECG, and after success CPR a progressive and almost complete restoration in MAP and HR, above all in epinephrine- plus NDP-α-MSH-treated animals (Fig. 1). Treatment of sham animals with i.v. epinephrine plus NDP-α-MSH did not affect MAP nor HR ($n=9$, not shown). Obviously, MAP and HR values, as well as all the following reported data, refer only to animals surviving at 3 h after CPR.

3.2. NDP-α-MSH restores basal conditions of hemogasanalysis parameters

In epinephrine-treated rats, CA/CPR caused significant changes in pH, PO_2, PCO_2, HCO_3^-, SBE and SO_2 values, as recorded at 1 h and 3 h after CPR (Table 2). At the same time points, significant alterations of Hct, lactate, Na^+ and Ca^{2+} blood levels were not found in our CA/CPR model; on the contrary, K^+ concentration increased in epinephrine-treated CA/CPR rats (seemingly due to KCl injection) (Table 2). Treatment with epinephrine plus NDP-α-MSH almost completely restored the basal values of hemogasanalysis parameters within 1 h after CPR, the values remaining stable up to the 3 h observation period (Table 2). Treatment of sham animals with i.v. epinephrine plus NDP-α-MSH did not alter hemogasanalysis parameters ($n=9$, not shown).

3.3. NDP-α-MSH activates JAK/STAT signaling in the heart

Western blot analysis at the end of the 3 h observation period showed, in the left ventricle of epinephrine-treated CA/CPR rats, a mild expression of the active pJAK2 and pTyr-STAT3 (which is pJAK2-dependent), but neither pERK1/2 nor pSer-STAT3 (pERK1/2-dependent) were affected (Fig. 2). In rats treated, during CPR, with epinephrine plus NDP-α-MSH there was a significantly increased expression of pJAK2 and pTyr-STAT3, relative to animals treated with epinephrine alone, whereas pERK1/2 and pSer-STAT3 were unaffected (Fig. 2).

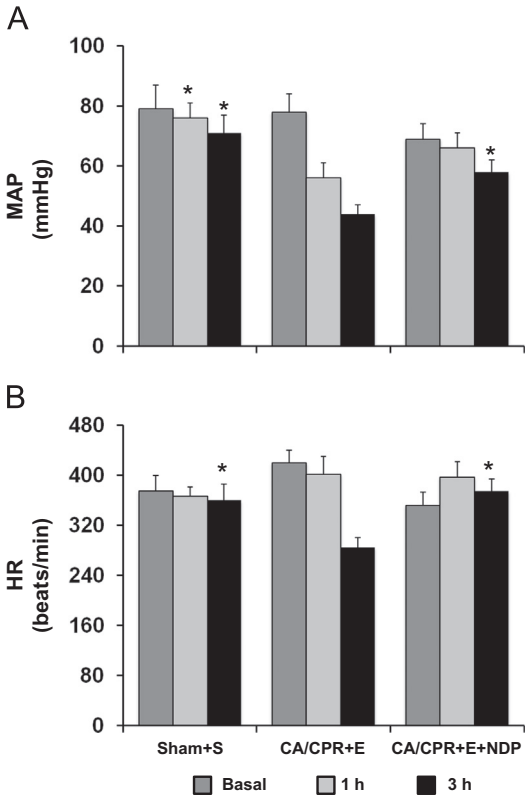

Fig. 1. Influence of NDP-α-MSH and epinephrine on mean arterial pressure (MAP) and heart rate (HR) restoration in rats subjected to cardiac arrest (CA) followed by cardiopulmonary resuscitation (CPR). Panel A: MAP in basal conditions, 1 h and 3 h after CPR; panel B: HR in basal conditions, 1 h and 3 h after CPR. Mean values ± S.E. M. for 7–10 rats per group. Sham = sham CA/CPR; *S* = saline; *E* = epinephrine (0.1 mg/kg i.v., 1 min after starting CPR); NDP = NDP-α-MSH (340 μg/kg i.v., 1 min after starting CPR). $^{*}P < 0.05$, at least, vs. the same time point of CA/CPR + epinephrine (ANOVA followed by Student–Newman–Keuls test).

3.4. NDP-α-MSH attenuates oxidative stress in the heart

To investigate CA/CPR-triggered oxidative stress in the left ventricle, 3 h after CPR we evaluated MDA concentration (as an index of oxidative stress-induced lipid peroxidation). In epinephrine-treated rats we detected a higher concentration of MDA, as compared with sham CA/CPR animals, whereas in CA/CPR rats treated with epinephrine plus NDP-α-MSH there was a marked reduction (Fig. 3).

3.5. NDP-α-MSH attenuates inflammatory response in the heart

Next, we explored the myocardial CA/CPR-induced inflammatory response by assessing the pro-inflammatory cytokines TNF-α and IL-6, as well as the anti-inflammatory cytokine IL-10. At the end of the 3 h period of return to spontaneous circulation, immunoblot analysis showed that in the left ventricle of epinephrine-treated rats there was a significant increase in TNF-α and IL-6 levels, and a significant decrease in IL-10 expression, as compared with sham CA/CPR animals (Fig. 4). In contrast, in CA/CPR rats treated with epinephrine plus NDP-α-MSH there was a marked reduction in TNF-α and IL-6 levels, whereas those of IL-10 increased (Fig. 4).

3.6. Apoptosis is not involved

Finally, at the end of the 3 h observation period, in the left ventricle we investigated apoptosis by assessing expression of BAX (that promotes apoptosis) and of the downstream executioner caspase-3 (for its specific involvement in DNA fragmentation).

Table 2
Effect of NDP-α-MSH and epinephrine on venous pH, PO_2, PCO_2, HCO_3^-, SBE, SO_2, lactate, Na^+, K^+, Ca^{2+} levels and Htc in rats subjected to CA/CPR.

Groups	Time	pH	PO_2 (mm Hg)	PCO_2 (mm Hg)	HCO_3^- (mEq/l)	SBE (mEq/l)	SO_2 (%)	Lactate (mmol/l)	Na^+ (mEq/l)	K^+ (mEq/l)	Ca^{2+} (mEq/l)	Htc (%)
Sham + saline	Basal	7.43 ± 0.03	50.83 ± 3.91	37.33 ± 3.8	24.3 ± 1.16	0.02 ± 1.00	88.5 ± 1.48	4.25 ± 0.68	134.33 ± 0.95	4.80 ± 0.45	2.25 ± 0.05	46.63 ± 0.86
	1 h	7.47 ± 0.02[a]	40.33 ± 3.64	29.83 ± 2.6[a]	21.53 ± 1.41	−2.13 ± 1.41[a]	77.17 ± 3.77[a]	4.82 ± 0.91	132.00 ± 1.15	3.87 ± 0.11[a]	2.43 ± 0.06	45.50 ± 1.36
	3 h	7.46 ± 0.03[a]	35.33 ± 3.01[a]	29.33 ± 2.47[a]	20.88 ± 1.63[a]	−2.95 ± 1.83[a]	70.00 ± 4.39[a]	3.92 ± 0.72	133.17 ± 1.38	4.62 ± 0.17[a]	2.32 ± 0.08	42.67 ± 0.71
CA/CPR + E	Basal	7.44 ± 0.03	46.20 ± 2.31	37.00 ± 4.99	24.42 ± 1.90	0.32 ± 1.37	83.75 ± 1.48	3.81 ± 0.83	138.40 ± 2.06	4.38 ± 0.33	2.24 ± 0.10	43.25 ± 0.92
	1 h	7.13 ± 0.10	33.60 ± 3.06	47.00 ± 3.88	17.20 ± 1.92	−10.58 ± 3.03	54.00 ± 4.36	4.45 ± 1.18	139.40 ± 1.36	6.75 ± 0.99	2.40 ± 0.05	43.10 ± 1.39
	3 h	7.14 ± 0.05	24.60 ± 1.23	41.20 ± 1.62	14.64 ± 0.72	−14.44 ± 3.07	33.25 ± 8.35	3.36 ± 0.81	136.40 ± 2.04	7.75 ± 2.22	2.46 ± 0.09	43.29 ± 1.90
CA/CPR + E + NDP	Basal	7.48 ± 0.02	43.20 ± 5.53	39.80 ± 6.22	26.04 ± 2.02	1.86 ± 1.55	76.80 ± 6.78	4.32 ± 0.61	137.60 ± 1.66	4.80 ± 0.17	2.38 ± 0.04	45.80 ± 1.46
	1 h	7.37 ± 0.04[a]	31.00 ± 1.52	35.00 ± 3.78[a]	19.70 ± 0.97	−5.64 ± 1.17	56.20 ± 4.71	4.88 ± 0.94	138.20 ± 1.88	4.70 ± 0.27[a]	2.36 ± 0.10	44.20 ± 1.43
	3 h	7.38 ± 0.02[a]	31.25 ± 0.55[a]	33.25 ± 2.78[a]	18.83 ± 1.72[a]	−6.60 ± 0.88[a]	54.50 ± 3.18[a]	3.75 ± 0.69	136.25 ± 1.60	4.55 ± 0.23[a]	2.48 ± 0.08	42.75 ± 1.65

Values are means ± S.E.M. ($n = 7$–10 rats per group) in basal conditions, 1 h and 3 h after sham procedure or CPR. CA = Cardiac Arrest; CPR = Cardiopulmonary resuscitation; sham = sham CA/CPR; HCO_3^- = bicarbonate; PCO_2 = carbon dioxide partial pressure; PO_2 = oxygen partial pressure; SBE = standard base excess; SO_2 = oxygen saturation; Htc = hematocrit; *E* = Epinephrine (0.1 mg/kg, i.v.); NDP = NDP-α-MSH (340 μg/kg, i.v.).

[a] $P < 0.05$, at least, vs. the corresponding value of CA/CPR + epinephrine (ANOVA followed by Student–Newman–Keuls test).

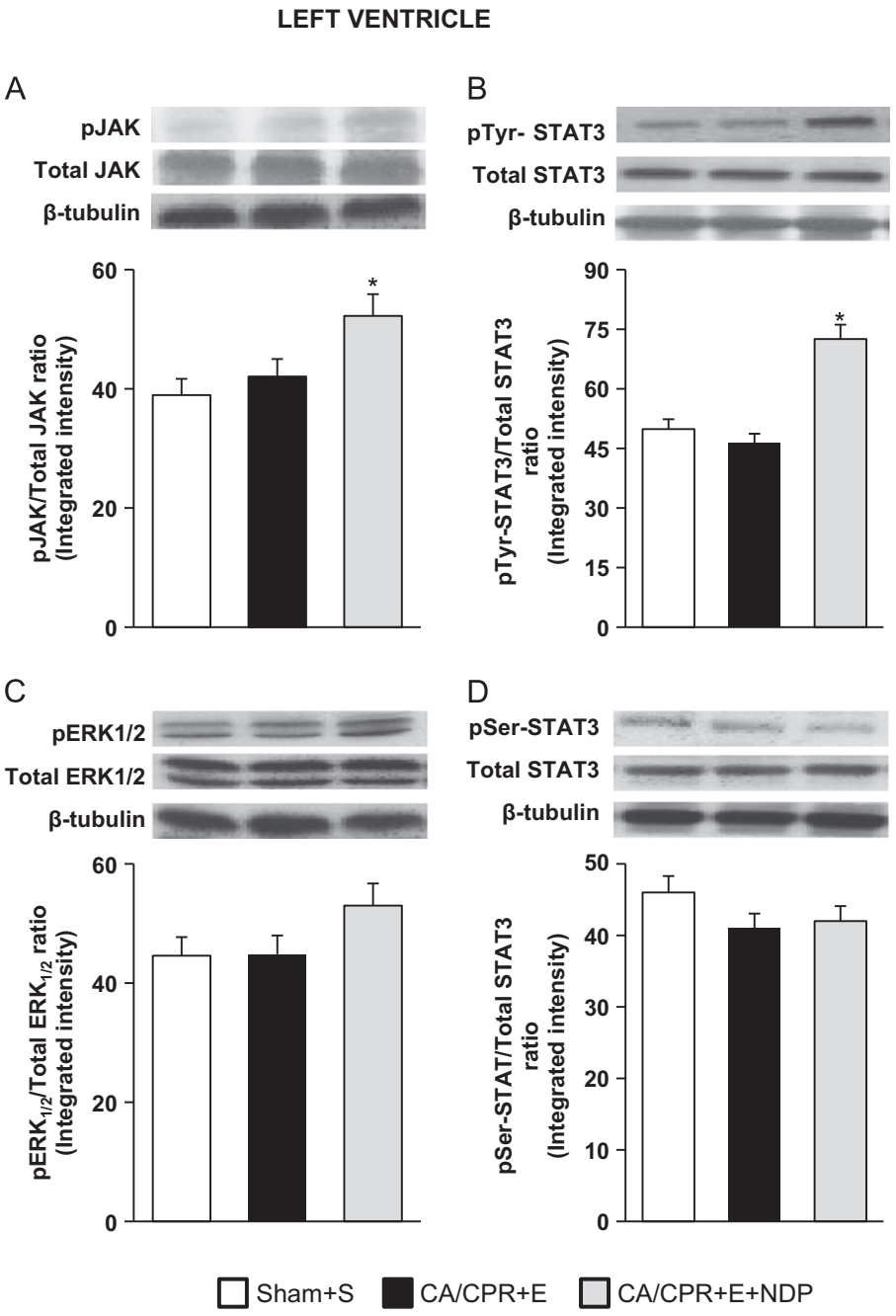

Fig. 2. Influence of NDP-α-MSH and epinephrine on the expression/activity of (A) janus kinases (pJAK/total JAK ratio), signal transducers and activators of transcription (B) pTyr-STAT3 (pTyr-STAT3/total STAT3 ratio) and (C) pSer-STAT3 (pSer-STAT3/total STAT3 ratio), and extracellular signal-regulated kinases ($pERK_{1/2}$/total $ERK_{1/2}$ ratio), in the left ventricle of rats subjected to cardiac arrest (CA) followed by cardiopulmonary resuscitation (CPR). The top of each panel shows representative immunoblots highlighting expression/activity of the respective marker(s) and house-keeping gene product β-tubulin. Means values ± S.E.M. for 7–10 rats per group, 3 h after CPR. Sham = sham CA/CPR; *S* = saline; *E* = epinephrine (0.1 mg/kg i.v., 1 min after starting CPR); NDP = NDP-α-MSH (340 μg/kg i.v., 1 min after starting CPR). $^{*}P < 0.05$, at least, vs. the corresponding value of CA/CPR + epinephrine (ANOVA followed by Student–Newman–Keuls test).

Immunoblot analysis showed no difference between sham and CA/CPR animals, irrespective of pharmacological treatment (Fig. 5).

4. Discussion

CA and CPR constitute a major problem both in- and out-of-hospital (Chang et al., 2007; Nichol et al., 2008; Peberdy et al., 2010; Rea et al., 2004; Thom et al., 2006). Routinely used vasopressors (e.g., vasopressin, epinephrine, or their combination), albeit have beneficial effects in the initial phase of CPR, exacerbate myocardial ischemia/reperfusion injury and fail to significantly improve survival and neurological outcome to hospital discharge (Lin et al., 2014; Yu et al., 2013). Novel pharmacological approaches able to ease CPR and also to minimize the general adverse consequences of CA are, therefore, needed (Brücken et al., 2014; Kida et al., 2012; Kurita et al., 2010; Lee et al., 2012; Norman et al., 2011; Parnia et al., 2014; Yu et al., 2013).

Here we show that in a rat model of CA followed by CPR plus epinephrine treatment, associated with significant free radical discharge and inflammatory reaction in the left ventricle, metabolic acidosis, hypotension, bradycardia and only 48% survival at the end of the 3 h observation period, i.v. concomitant treatment during CPR with the melanocortin analog NDP-α-MSH almost

completely restores the basal conditions of MAP and HR, reverses hypoxemia and metabolic acidosis, induces left ventricle expression of the protective active transcription factors pJAK2 and pTyr-STAT3, and improves survival rate. We also show that under NDP-α-MSH treatment oxidative stress is diminished by melanocortin treatment, as indicated by the reduced levels of MDA in the left ventricle. Accordingly, melanocortins have been repeatedly reported to reduce blood levels of oxygen-derived free radicals and nitric oxide under pre-terminal conditions such as myocardial ischemia/reperfusion, circulatory shock and respiratory arrest (Giuliani et al., 2007a; Guarini et al., 1996, 1997a, 1997b, 1998; Mioni et al., 2003, 2005), that could likewise contribute to prevent organ damage in CA/CPR. The cardioprotective JAK/ERK/STAT signaling pathways are also thought to supervise the transcriptional regulation of several genes that modulate inflammation (Bolli et al., 2011; de Jong et al., 2012; Minamino, 2012; Obana et al., 2012; Ottani et al., 2013; Xuan et al., 2001, 2005). Consistently, here we report that co-administration of epinephrine and NDP-α-MSH also reduces heart levels of the pro-inflammatory mediators TNF-α and IL-6, whereas increases those of the anti-inflammatory cytokine IL-10. On the contrary, apoptosis is not affected in our experimental model (probably because of the short period of CA; see also below): indeed, no difference we found in the expression of BAX and caspase-3 between sham and CA/CPR animals, irrespective of pharmacological treatment.

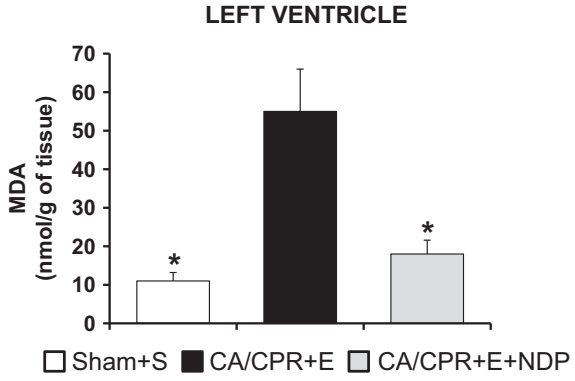

Fig. 3. Influence of NDP-α-MSH and epinephrine on malondialdeyde (MDA) levels in the left ventricle of rats subjected to cardiac arrest (CA) followed by cardiopulmonary resuscitation (CPR). Mean values ± S.E.M. for 6 rats per group, 3 h after CPR. Sham = sham CA/CPR; *S* = saline; *E* = epinephrine (0.1 mg/kg i.v., 1 min after starting CPR); NDP = NDP-α-MSH (340 μg/kg i.v., 1 min after starting CPR). *$P < 0.001$ vs. CA/CPR + epinephrine (ANOVA followed by Student–Newman–Keuls test).

We previously reported that melanocortins up-regulate expression of pERK1/2 in experimental models of myocardial ischemia/reperfusion in Wistar rats (Mioni et al., 2005; Ottani et al., 2010, 2013). Surprisingly, in our experimental conditions of CA/CPR (Sprague–Dawley, older rats), heart injury – irrespective of treatment type – did not affect expression of pERK1/2, nor of pSer-STAT3 (pERK1/2-dependent). These findings suggest that pERK1/2 might be involved as anti-apoptotic pro-survival kinases in pathophysiological conditions different from CA/CPR, the discrepancy maybe also depending on animal strain and age: consistently, in our study we did not detect apoptotic response. Further, and according to our present data, activation of the classic pro-survival kinases ERK1/2, although considered important, is thought not essential for cardioprotection against reperfusion-induced damage, as demonstrated by using different models of animal species, because alternative (STAT3-dependent) protective pathways exist (Lecour et al., 2005).

Obviously, our experimental model of CA/CPR is characterized by a marked alterations of hemogasanalysis parameters. The restored values of venous PO_2 and SO_2, here shown, indicate that melanocortins are able to improve circulatory functions, and those of PCO_2, pH, HCO_3^- and SBE reflect a reversal of metabolic acidosis, according to previous studies carried out in other hypoxic conditions such as circulatory shock (for review see: Giuliani et al., 2010, 2012). Of interest, lactate levels markedly increase only during CA (Kurita et al., 2010), and normal blood concentration is usually reached within 1 h after return to spontaneous circulation, in agreement with our present results. Significant changes of Hct, and electrolyte blood levels such as Na^+ and Ca^{2+}, did not occur in our CA/CPR model; on the contrary, K^+ concentration increased in CA/CPR rats treated with epinephrine alone (seemingly due to KCl injection), and the capability of NDP-α-MSH to restore K^+ level could reflect a preservation of renal function.

Overall, our results – obtained by evaluating return to spontaneous circulation, survival, hemogasanalysis parameters, oxidative stress, and biomolecular changes – suggest that treatment of CA/

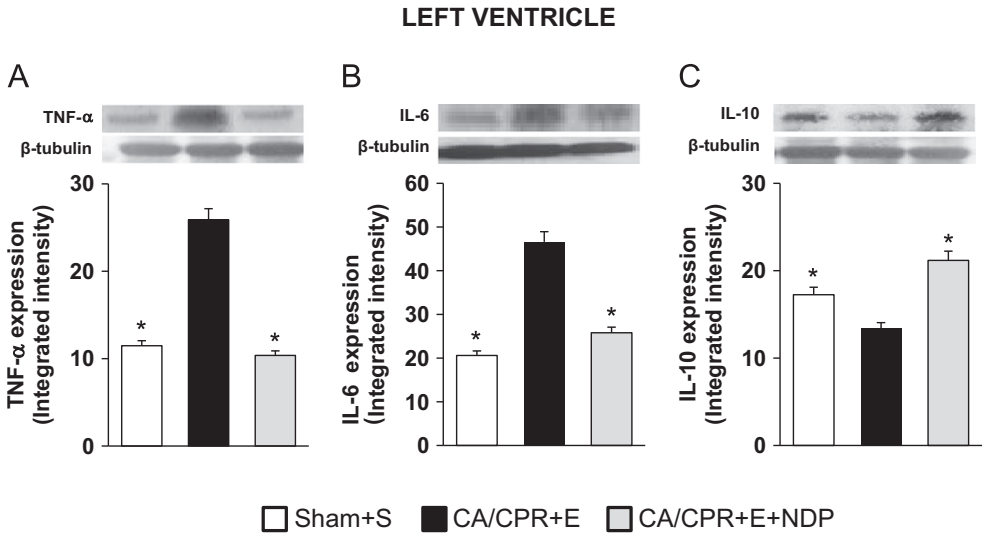

Fig. 4. Influence of NDP-α-MSH and epinephrine on the expression of (A) tumor necrosis factor-α (TNF-α), (B) interleukin-6 (IL-6) and (C) IL-10, in the left ventricle of rats subjected to cardiac arrest (CA) followed by cardiopulmonary resuscitation (CPR). The top of each panel shows representative immunoblots highlighting expression of the respective marker and house-keeping gene product β-tubulin. Mean values ± S.E.M. for 7–10 rats per group, 3 h after CPR. Sham = sham CA/CPR;; *S* = saline; *E* = epinephrine (0.1 mg/kg i.v., 1 min after starting CPR); NDP = NDP-α-MSH (340 μg/kg i.v., 1 min after starting CPR). *$P < 0.01$, at least, vs. the corresponding value of CA/CPR + epinephrine (ANOVA followed by Student–Newman–Keuls test).

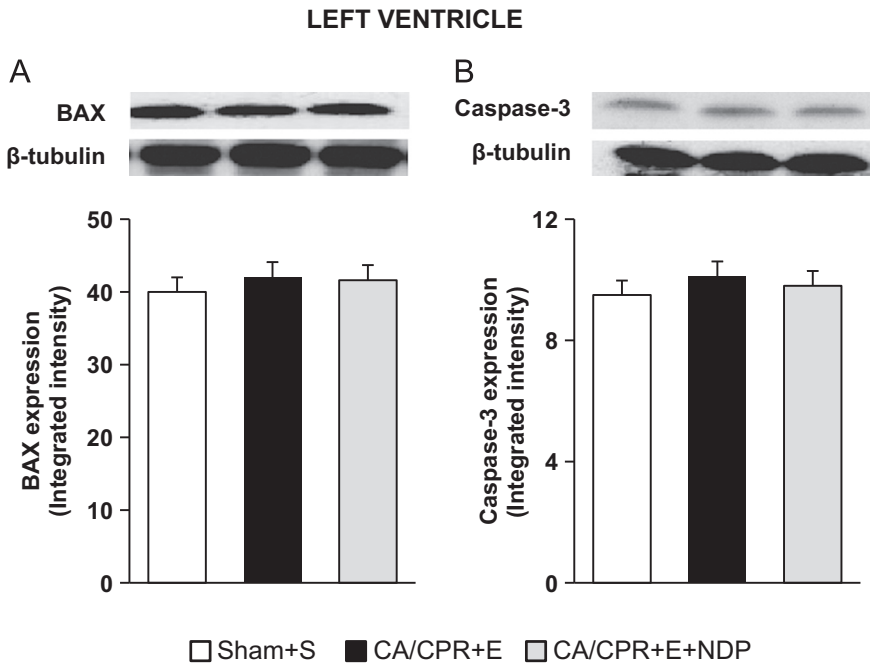

Fig. 5. Influence of NDP-α-MSH and epinephrine on the expression of (A) BAX and (B) caspase-3 in the left ventricle of rats subjected to cardiac arrest (CA) followed by cardiopulmonary resuscitation (CPR). The top of each panel shows representative immunoblots highlighting expression of the respective marker and house-keeping gene product β-tubulin. Means values ± S.E.M. for 7–10 rats per group, 3 h after CPR. Sham = sham CA/CPR; *S* = saline; *E* = epinephrine (0.1 mg/kg i.v., 1 min after starting CPR); NDP = NDP-α-MSH (340 μg/kg i.v., 1 min after starting CPR). $P > 0.05$ among groups (ANOVA).

CPR with a combination of epinephrine and NDP-α-MSH could be cardioprotective and more effective than epinephrine alone. This is in agreement with the idea that epinephrine may exacerbate CA/CPR-induced heart dysfunction as a consequence of increased myocardial oxygen demand (Lin et al., 2014; Yu et al., 2013).

Melanocortins have been shown to induce multi-organ protection in several experimental models of hypoxic conditions, including circulatory shock, respiratory arrest, brain ischemia, myocardial ischemia and more, and many investigations (both past and recent) suggest that these protective effects result from brain melanocortin MC_3/MC_4 receptor-activated anti-inflammatory mechanisms, mediated by the vagus nerve (Bazzani et al., 2002; Bertolini et al., 1989; Getting et al., 2006; Giuliani et al., 2007a, 2007b, 2012; Guarini et al., 1997a; Mioni et al., 2003, 2005; Ottani et al., 2010, 2013). Indeed, melanocortins pass the blood-brain barrier in pharmacologically relevant concentration (Catania et al., 2004; Giuliani et al., 2012), and in previous studies with experimental models of short-term and prolonged myocardial ischemia/reperfusion we demonstrated that melanocortins induce cardioprotection through a brain activation of an efferent vagal fibre-mediated cholinergic anti-inflammatory pathway (Mioni et al., 2005; Ottani et al., 2010). Noteworthy, the vagus nerve-mediated cholinergic anti-inflammatory pathway – which is thought to represent a rapid, self-defence neural mechanism, and is activated by melanocortins (Giuliani et al., 2010, 2012; Guarini et al., 2003; Mioni et al., 2005; Ottani et al., 2010; Tracey, 2002) – has been reported to inhibit macrophage inflammatory responses in a mouse model of intestinal manipulation by activating the JAK2/STAT3 signaling (de Jonge et al., 2005). Further, in mice it has been shown that CA/CPR impairs the cholinergic anti-inflammatory pathway (Norman et al., 2011). Overall these findings support our present data, and also suggest that in experimental conditions of CA/CPR injury melanocortin-induced molecular changes, including attenuation of free radical discharge and inflammatory response, in the acute phase of the disease likely play a key protective role not only in the heart, but also in the brain, in agreement with the established neuroprotective effects of melanocortin analogs (Gatti et al., 2012; Giuliani et al., 2006, 2007b, 2009), and probably via activation of the cholinergic anti-inflammatory pathway. On the basis of previous studies performed in other hypoxic conditions (Giuliani et al., 2012), it can be hypothesized that other organs could benefit from a melanocortin treatment in CA/CPR.

It is well known that melanocortins modulate inflammatory responses by down-regulating the expression of pro-inflammatory mediators and by up-regulating that of anti-inflammatory factors, including the potent anti-inflammatory cytokine IL-10 (Catania et al., 2004; Giuliani et al., 2012), and IL-10 has been reported to reduce heart damage in conditions of acute myocardial infarction via activation of STAT3 (Frangogiannis, 2012; Krishnamurthy et al., 2009). A growing body of evidence indicates that the anti-inflammatory/cardioprotective effects of melanocortins are adrenal-independent (Catania et al., 2004; Giuliani et al., 2010, 2012). In the present investigation we used a melanocortin receptor agonist, NDP-α-MSH, that binds melanocortin MC_1, MC_3, MC_4 and MC_5 receptors, but not MC_2 receptors, which are expressed in adrenal cortex and mediate glucocorticoid release (Catania et al., 2004; Giuliani et al., 2012; Wikberg and Mutulis, 2008); thus, an involvement of adrenal glands can be ruled out also in the effects here reported. Further, on the basis of our previous studies (Ottani et al., 2013), we rule out a direct activation of STAT3. The multiple beneficial effects against CA/CPR found in our present study, including activation of STAT3, might be due to a physiologically arranged self-defence machinery that is activated after melanocortin receptor-mediated signal transduction of NDP-α-MSH, and that targets multiple CA/CPR-related pathophysiological pathways. Not even the anti-radical effect here shown can be ascribed to a direct radical scavenging activity of melanocortins, as previously demonstrated by competition experiments (reviewed by: Giuliani et al., 2007a, 2010, 2012). Obviously, studies on the role of specific melanocortin receptor subtypes in CA/CPR are useful, and we have planned further research in this direction.

In conclusion, these results indicate that, in experimental CA/CPR, melanocortins are able to improve return to spontaneous circulation, to reverse metabolic acidosis and to inhibit heart free

radical discharge and inflammatory cascade, likely via activation of the JAK2/STAT3 signaling pathway. Overall, these findings could reflect a melanocortin-induced reduction of heart susceptibility to ischemia/reperfusion injury, via JAK2/STAT3 signaling: indeed, the JAK/STAT pathway plays an important role in pre- and post-conditioning (Bolli et al., 2011; Obana et al., 2012; Xuan et al., 2001). Moreover, melanocortins have established antipyretic effects (Catania et al., 2004; Giuliani et al., 2012), that could be clinically relevant: it is well known, in fact, that therapeutic hypothermia has a protective action in CA/CPR (Lee et al., 2012). Therefore, in CA/CPR conditions melanocortins, co-administered with epinephrine, could be promising drugs for a treatment able to rapidly improve cardiovascular function and tissue perfusion and to block the main pathophysiological mechanisms of organ damage. Further, melanocortins are devoid of appreciable toxicity (Giuliani et al., 2012; Wikberg and Mutulis, 2008), and the resuscitating and protective effects of melanocortin analogs in experimental and clinical conditions of circulatory shock, myocardial ischemia and cerebral ischemia have been recently highlighted to clinicians by Corander et al. (2009). The present findings should encourage further studies – in particular long-term studies are needed – because a treatment with melanocortins plus epinephrine might represent a novel, safe and non-toxic approach to the management of CA/CPR and consequent detrimental systemic responses, including neurological, renal, hepatic and pulmonary dysfunctions.

Acknowledgments

Supported, in part, by a grant (E91J08000630005) from Department of Biomedical, Metabolic and Neural Sciences of University of Modena and Reggio Emilia, Italy.

References

Angelos, M.G., Yeh, S.T., Aune, S.E., 2011. Post-cardiac arrest hyperoxia and mitochondrial function. Resuscitation 82 (Suppl 2), S48–S51.
Bazzani, C., Mioni, C., Ferrazza, G., Cainazzo, M.M., Bertolini, A., Guarini, S., 2002. Involvement of the central nervous system in the protective effect of melanocortins in myocardial ischaemia/reperfusion injury. Resuscitation 52, 109–115.
Bertolini, A., Ferrari, W., Guarini, S., 1989. The adrenocorticotropic hormone (ACTH)-induced reversal of hemorrhagic shock. Resuscitation 18, 253–267.
Bolli, R., Stein, A.B., Guo, Y., Wang, O.L., Rokosh, G., Dawn, B., Molkentin, J.D., Sanganalmath, S.K., Zhu, Y., Xuan, Y.T., 2011. A murine model of inducible, cardiac-specific deletion of STAT3: its use to determine the role of STAT3 in the upregulation of cardioprotective proteins by ischemic preconditioning. J. Mol. Cell. Cardiol. 50, 589–597.
Brücken, A., Kurnaz, P., Bleilevens, C., Derwall, M., Weis, J., Nolte, K., Rossaint, R., Fries, M., 2014. Dose dependent neuroprotection of the noble gas argon after cardiac arrest in rats is not mediated by KATP-Channel opening. Resuscitation 85, 826–832.
Catania, A., Gatti, S., Colombo, G., Lipton, J.M., 2004. Targeting melanocortin receptors as a novel strategy to control inflammation. Pharmacol. Rev. 56, 1–29.
Chang, W.T., Ma, M.H., Chien, K.L., Huang, C.H., Tsai, M.S., Shih, F.Y., Yuan, A., Tsai, K. C., Lin, F.Y., Lee, Y.T., Chen, W.J., 2007. Postresuscitation myocardial dysfunction: correlated factors and prognostic implications. Intensive Care Med. 33, 88–95.
Chalkias, A., Xanthos, T., 2012. Redox-mediated programed death of myocardial cells after cardiac arrest and cardiopulmonary resuscitation. Redox Rep. 17, 80–83.
Corander, M.P., Fenech, M., Coll, A.P., 2009. Science of self-preservation: how melanocortin action in the brain modulates body weight, blood pressure and ischemic damage. Circulation 120, 2260–2268.
de Jong, P.R., Schadenberg, A.W., van den Broek, T., Beekman, J.M., van Wijk, F., Coffer, P.J., Prakken, B.J., Jansen, N.J., 2012. STAT3 regulates monocyte TNF-alpha production in systemic inflammation caused by cardiac surgery with cardiopulmonary bypass. PLoS One 7, e35070.
de Jonge, W.J., van der Zanden, E.P., The, F.O., Bijlsma, M.F., van Westerloo, D.J., Bennink, R.J., Berthoud, H.R., Uematsu, S., Akira, S., van den Wijngaard, R.M., Boeckxstaens, G.E., 2005. Stimulation of the vagus nerve attenuates macrophage activation by activating the Jak2-STAT3 signaling pathway. Nat. Immunol. 6, 844–851.
Frangogiannis, N.G., 2012. Regulation of the inflammatory response in cardiac repair. Circ. Res. 110, 159–173.
Gatti, S., Lonati, C., Acerbi, F., Sordi, A., Leonardi, P., Carlin, A., Gaini, S.M., Catania, A., 2012. Protective action of NDP-MSH in experimental subarachnoid hemorrhage. Exp. Neurol. 234, 230–238.
Getting, S.J., Di Filippo, C., D'Amico, M., Perretti, M., 2006. The melanocortin peptide HP228 displays protective effects in acute models of inflammation and organ damage. Eur. J. Pharmacol. 532, 138–144.
Giuliani, D., Bitto, A., Galantucci, M., Zaffe, D., Ottani, A., Irrera, N., Neri, L., Cavallini, G.M., Altavilla, D., Botticelli, A.R., Squadrito, F., Guarini, S., 2014. Melanocortins protect against progression of Alzheimer's disease in triple-transgenic mice by targeting multiple pathophysiological pathways. Neurobiol. Aging 35, 537–547.
Giuliani, D., Minutoli, L., Ottani, A., Spaccapelo, L., Bitto, A., Galantucci, M., Altavilla, D., Squadrito, F., Guarini, S., 2012. Melanocortins as potential therapeutic agents in severe hypoxic conditions. Front. Neuroendocrinol. 33, 179–193.
Giuliani, D., Mioni, C., Altavilla, D., Leone, S., Bazzani, C., Minutoli, L., Bitto, A., Cainazzo, M.M., Marini, H., Zaffe, D., Botticelli, A.R., Pizzala, R., Savio, M., Necchi, D., Schiöth, H.B., Bertolini, A., Squadrito, F, Guarini, S., 2006. Both early and delayed treatment with melanocortin 4 receptor-stimulating melanocortins produces neuroprotection in cerebral ischemia. Endocrinology 147, 1126–1135.
Giuliani, D., Mioni, C., Bazzani, C., Zaffe, D., Botticelli, A.R., Capolongo, S., Sabba, A., Galantucci, M., Iannone, A., Grieco, P., Novellino, E., Colombo, G., Tomasi, A., Catania, A., Guarini, S., 2007a. Selective melanocortin MC_4 receptor agonists reverse haemorrhagic shock and prevent multiple organ damage. Br. J. Pharmacol. 150, 595–603.
Giuliani, D., Ottani, A., Altavilla, D., Bazzani, C., Squadrito, F., Guarini, S., 2010. Melanocortins and the cholinergic anti-inflammatory pathway. Adv. Exp. Med. Biol. 681, 71–87.
Giuliani, D., Ottani, A., Minutoli, L., Di Stefano, V., Galantucci, M., Bitto, A., Zaffe, D., Altavilla, D., Botticelli, A.R., Squadrito, F., Guarini, S., 2009. Functional recovery after delayed treatment of ischemic stroke with melanocortins is associated with overexpression of the activity-dependent gene Zif268. Brain Behav. Immun. 23, 844–859.
Giuliani, D., Ottani, A., Mioni, C., Bazzani, C., Galantucci, M., Minutoli, L., Bitto, A., Zaffe, D., Botticelli, A.R., Squadrito, F., Guarini, S., 2007b. Neuroprotection in focal cerebral ischemia owing to delayed treatment with melanocortins. Eur. J. Pharmacol. 570, 57–65.
Guarini, S., Bazzani, C., Bini, A., Cainazzo, M.M., Tomasi, A., Bertolini, A., 1998. Adrenocorticotropin counteracts the increase in free radical blood levels, detected by electron spin resonance spectrometry, in rats subjected to prolonged asphyxia. Life Sci. 63, 97–104.
Guarini, S., Bazzani, C., Mattera Ricigliano, G., Bini, A., Tomasi, A., Bertolini, A., 1996. Influence of ACTH-(1-24) on free radical levels in the blood of haemorrhage-shocked rats: direct ex vivo detection by electron spin resonance spectrometry. Br. J. Pharmacol. 119, 29–34.
Guarini, S., Altavilla, D., Cainazzo, M.M., Giuliani, D., Bigiani, A., Marini, H., Squadrito, G., Minutoli, L., Bertolini, A., Marini, R., Adamo, E.B., Venuti, F.S., Squadrito, F., 2003. Efferent vagal fibre stimulation blunts NF-*k*B activation and protects against hypovolemic hemorrhagic shock. Circulation 107, 1189–1194.
Guarini, S., Bazzani, C., Bertolini, A., 1997a. Resuscitating effect of melanocortin peptides after prolonged respiratory arrest. Br. J. Pharmacol. 121, 1454–1460.
Guarini, S., Bini, A., Bazzani, C., Mattera Ricigliano, G., Cainazzo, M.M., Tomasi, A., Bertolini, A., 1997b. Adrenocorticotropin normalizes the blood levels of nitric oxide in hemorrhage-shocked rats. Eur. J. Pharmacol. 336, 15–21.
Kida, K., Minamishima, S., Wang, H., Ren, J., Yigitkanli, K., Nozari, A., Mandeville, J.B., Liu, P.K., Liu, C.H., Ichinose, F., 2012. Sodium sulfide prevents water diffusion abnormality in the brain and improves long term outcome after cardiac arrest in mice. Resuscitation 83, 1292–1297.
Krishnamurthy, P., Rajasingh, J., Lambers, E., Qin, G., Losordo, D.W., Kishore, R., 2009. IL-10 inhibits inflammation and attenuates left ventricular remodeling after myocardial infarction via activation of STAT3 and suppression of HuR. Circ. Res. 104, E9–E18.
Kurita, A., Taniguchi, T., Yamamoto, K., 2010. The effects of carvedilol administration on cardiopulmonary resuscitation in a rat model of cardiac arrest induced by airway obstruction. Anesth. Analg. 111, 1207–1210.
Lecour, S., Suleman, N., Deuchar, G.A., Somers, S., Lacerda, L., Huisamen, B., Opie, L. H., 2005. Pharmacological preconditioning with tumor necrosis factor-alpha activates signal transducer and activator of transcription-3 at reperfusion without involving classic prosurvival kinases (Akt and extracellular signal-regulated kinase). Circulation 112, 3911–3918.
Lee, J.H., Suh, G.J., Kwon, W.Y., Kim, K.S., Rhee, J.E., Kim, M.A., Park, M.H., 2012. Protective effects of therapeutic hypothermia in post-resuscitation myocardium. Resuscitation 83, 633–639.
Lin, S., Callaway, C.W., Shah, P.S., Wagner, J.D., Beyene, J., Ziegler, C.P., Morrison, L.J., 2014. Adrenaline for out-of-hospital cardiac arrest resuscitation: a systematic review and meta-analysis of randomized controlled trials. Resuscitation 85, 732–740.
Meybohm, P., Gruenewald, M., Albrecht, M., Zacharowski, K.D., Lucius, R., Zitta, K., Koch, A., Tran, N., Scholz, J., Bein, B., 2009. Hypothermia and postconditioning after cardiopulmonary resuscitation reduce cardiac dysfunction by modulating inflammation, apoptosis and remodeling. PLoS One 4, e7588.
Minamino, T., 2012. Cardioprotection from ischemia/reperfusion injury: basic and translational research. Circ. J. 76, 1074–1082.
Mioni, C., Bazzani, C., Giuliani, D., Altavilla, D., Leone, S., Ferrari, A., Minutoli, L., Bitto, A., Marini, H., Zaffe, D., Botticelli, A.R., Iannone, A., Tomasi, A., Bigiani, A., Bertolini, A., Squadrito, F., Guarini, S., 2005. Activation of an efferent cholinergic pathway produces strong protection against myocardial ischemia/reperfusion injury in rats. Crit. Care Med. 33, 2621–2628.

Mioni, C., Giuliani, D., Cainazzo, M.M., Leone, S., Bazzani, C., Grieco, P., Novellino, E., Tomasi, A., Bertolini, A., Guarini, S., 2003. Further evidence that melanocortins prevent myocardial reperfusion injury by activating melanocortin MC_3 receptors. Eur. J. Pharmacol. 477, 227–234.

Nichol, G., Thomas, E., Callaway, C.W., Hedges, J., Powell, J.L., Aufderheide, T.P., Rea, T., Lowe, R., Brown, T., Dreyer, J., Davis, D., Idris, A., Stiell, I., 2008. Resuscitation outcomes consortium investigators. Regional variation in out-of-hospital cardiac arrest incidence and outcome. JAMA 300, 1423–1431.

Norman, G.J, Morris, J.S., Karelina, K., Weil, Z.M., Zhang, N., Al-Abed, Y., Brothers, H. M., Wenk, G.L., Pavlov, V.A., Tracey, K.J., Devries, A.C., 2011. Cardiopulmonary arrest and resuscitation disrupts cholinergic anti-inflammatory processes: a role for cholinergic α7 nicotinic receptors. J. Neurosci. 31, 3446–3452.

Obana, M., Miyamoto, K., Murasawa, S., Iwakura, T., Hayama, A., Yamashita, T., Shiragaki, M., Kumagai, S., Miyawaki, A., Takewaki, K., Matsumiya, G., Maeda, M., Yoshiyama, M., Nakayama, H., Fujio, Y., 2012. Therapeutic administration of IL-11 exhibits the post-conditioning effects against ischemia reperfusion injury via STAT3 in the heart. Am. J. Physiol. Heart Circ.Physiol 303, H569–77.

Ornato, J.P., 2008. Optimal vasopressor drug therapy during resuscitation. Crit. Care 12, 123.

Ottani, A., Giuliani, D., Galantucci, M., Spaccapelo, L., Novellino, E., Grieco, P., Jochem, J., Guarini, S., 2010. Melanocortins counteract inflammatory and apoptotic responses to prolonged myocardial ischemia/reperfusion through a vagus nerve-mediated mechanism. Eur. J. Pharmacol. 637, 124–130.

Ottani, A., Galantucci, M., Ardimento, E., Neri, L., Canalini, F., Calevro, A., Zaffe, D., Novellino, E., Grieco, P., Giuliani, D., Guarini, S., 2013. Modulation of the JAK/ERK/STAT signaling in melanocortin-induced inhibition of local and systemic responses to myocardial ischemia/reperfusion. Pharmacol. Res. 72, 1–8.

Paradis, N.A., Martin, G.B., Rosenberg, J., Rivers, E.P., Goetting, M.G., Appleton, T.J., Feingold, M., Cryer, P.E., Wortsman, J., Nowak, R.M., 1991. The effect of standard- and high-dose epinephrine on coronary perfusion pressure during prolonged cardiopulmonary resuscitation. JAMA 265, 1139–1144.

Parnia, S., Nasir, A., Ahn, A., Malik, H., Yang, J., Zhu, J., Dorazi, F., Richman, P., 2014. A feasibility study of cerebral oximetry during in-hospital mechanical and manual cardiopulmonary resuscitation. Crit. Care Med. 42, 930–933.

Peberdy, M.A., Callaway, C.W., Neumar, R.W., Geocadin, R.G., Zimmerman, J.L., Donnino, M., Gabrielli, A., Silvers, S.M., Zaritsky, A.L., Merchant, R., Vanden Hoek, T.L., Kronick, S.L., 2010. American Heart AssociationPart 9: post-cardiac arrest care: American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation 122, S768–786.

Rea, T.D., Eisenberg, M.S., Sinibaldi, G., White, R.D., 2004. Incidence of EMS-treated out-of-hospital cardiac arrest in the United States. Resuscitation 63, 17–24.

Thom, T., Haase, N., Rosamond, W., Howard, V.J., Rumsfeld, J., Manolio, T., Zheng, Z. J., Flegal, K., O'Donnell, C., Kittner, S., Lloyd-Jones, D., Goff Jr., D.C., Hong, Y., Adams, R., Friday, G., Furie, K., Gorelick, P., Kissela, B., Marler, J., Meigs, J., Roger, V., Sidney, S., Sorlie, P., Steinberger, J., Wasserthiel-Smoller, S., Wilson, M., Wolf, P., 2006. American Heart Association Statistics Committee and Stroke Statistics SubcommitteeHeart disease and stroke statistics–update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 113, e85–151.

Tracey, K.J., 2002. The inflammatory reflex. Nature 420, 853–859.

Wikberg, J. E, Mutulis, F., 2008. Targeting melanocortin receptors: an approach to treat weight disorders and sexual dysfunction. Nat. Rev. Drug Discov. 7, 307–323.

Xuan, Y.T., Guo, Y., Han, H., Zhu, Y., Bolli, R., 2001. An essential role of the JAK-STAT pathway in ischemic preconditioning. Proc. Natl. Acad. Sci. USA 98, 9050–9055.

Xuan, Y.T., Guo, Y., Zhu, Y., Wang, O.L., Rokosh, G., Messing, R.O., Bolli, R., 2005. Role of the protein kinase C-ε-Raf-1-MEK-1/2-p44/42 MAPK signaling cascade in the activation of signal transducers and activators of transcription 1 and 3 and induction of cyclooxygenase-2 after ischemic preconditioning. Circulation 112, 1971–1978.

Yu, H., Qing, H., Lei, Z., 2013. Cytidine diphosphate choline improves the outcome of cardiac arrest vs epinephrine in rat model. Am. J. Emerg. Med. 31, 1022–1028.