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Further evidence that melanocortins prevent myocardial reperfusion injury by activating melanocortin MC_3 receptors

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Abstract

In rats subjected to myocardial ischemia/reperfusion, melanocortin peptides, including γ_1-melanocyte-stimulating hormone (γ_1-MSH), are able to exert a protective effect by stimulating brain melanocortin MC_3 receptors. A non-melanocortin receptor belonging to a group of receptors for Phe-Met-Arg-Phe-NH_2 (FMRFamide)-like peptides may be involved in some of the cardiovascular effects of the γ-MSHs. FMRFamide-like peptides and γ_1-/γ_2-MSH share, among other things, the C-terminal Arg-Phe sequence, which seems to be essential for cardiovascular effects in normal animals. So we aimed to further investigate which receptor and which structure are involved in the protective effects of melanocortins in anesthetized rats subjected to myocardial ischemia by ligature of the left anterior descending coronary artery (5 min), followed by reperfusion. In saline-treated rats, reperfusion induced, within a few seconds, a high incidence of ventricular tachycardia and ventricular fibrillation, and a high percentage of death within the 5 min of observation period. Reperfusion was associated with a massive increase in free radical blood levels and with an abrupt and marked fall in systemic arterial pressure. The i.v. treatment (162 nmol/kg) during the ischemic period with the adrenocorticotropin fragment 1–24 [ACTH-(1–24): the reference protective melanocortin which binds all melanocortin receptors], as well as with both the melanocortin MC_3 receptor agonists γ_2-MSH and [D-Trp^8]γ_2-MSH, reduced the incidence of ventricular tachycardia, ventricular fibrillation and death, the increase in free radical blood levels and the fall in arterial pressure. On the contrary, γ_2-MSH-(6–12) (a fragment unable to bind melanocortin receptors) was ineffective. Such protective effect was prevented by the melanocortin MC_3/MC_4 receptor antagonist SHU 9119. In normal (i.e., not subjected to myocardial ischemia/reperfusion) rats, the same i.v. dose (162 nmol/kg) of γ_2-MSH, [D-Trp^8]γ_2-MSH and γ_2-MSH-(6–12) provoked a prompt and transient increase in arterial pressure; on the other hand, ACTH-(1–24), which lacks the C-terminal Arg-Phe sequence, decreased arterial pressure, but only at higher doses. Heart rate of normal rats was not affected by any of the assayed peptides. The present data confirm and extend our previous findings that melanocortins prevent myocardial reperfusion injury by activating melanocortin MC_3 receptors. Moreover, they further support the notion that, in normal rats, cardiovascular effects of γ-MSHs are mediated by receptors for FMRFamide-like peptides, for whose activation, but not for that of melanocortin MC_3 receptors, the C-terminal Arg-Phe structure being relevant.

Keywords: Myocardium; Reperfusion injury; Oxygen free radical; Melanocortin MC_3 receptor; γ-MSH (γ-melanocyte-stimulating hormone); FMRFamide-like (Phe-Met-Arg-Phe-NH_2-like) peptide

1. Introduction

Myocardial reperfusion after a transient period of coronary occlusion induces the occurrence of a high incidence of ventricular tachycardia and ventricular fibrillation, and a high lethality rate (Manning and Hearse, 1984). The main pathogenetic factors of such sudden arrhythmogenesis include the oxygen free radical discharge during reperfusion (Bernier et al., 1986; Bolli and Marban, 1999), with some controversy (Yamada et al., 1990).

Several mechanisms are involved in the generation of oxygen free radicals (Ar'Rajab et al., 1996). Among others,

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the reduction of molecular oxygen by the xanthine oxidase is of crucial importance (Reilly et al., 1991). Myocardial ischemia induces, with species-related differences, a massive release of noradrenaline from cardiac sympathetic nerve endings, which causes generation of oxygen free radicals (Kurz et al., 1996; Obata and Yamanaka, 1999; Schömig et al., 1984; Seyfarth et al., 1993). This contributes both to the occurrence of severe ventricular arrhythmias and to the development of irreversible cellular damage (Rona, 1985). Moreover, it has been reported that endothelin-mediated overproduction of free radicals mediates the post-ischemic endothelial dysfunction in the heart (Maczewski and Beresewicz, 2000).

A lot of experimental evidence indicates that this transient functional abnormality of the heart is associated with an inflammatory response (Frangogiannis et al., 2002; Lucchesi, 1990). Oxygen free radicals may be involved in triggering inflammatory cascade through the activation of nuclear factor-kappa B (NF-κB) and consequent induction of cytokine synthesis, including tumor necrosis factor-α (TNF-α) (Altavilla et al., 2000; Chandrasekar and Freeman, 1997; Dhalla et al., 2000; Kupatt et al., 1999; Lefer and Granger, 2000).

Several melanocortin peptides [adrenocorticotropic hormone (ACTH), α-melanocyte-stimulating hormone (α-MSH) and other fragments lacking the C-terminal Arg-Phe sequence] have a life-saving effect in animals and humans in conditions of severe tissue hypoxia as occurs during shock states (Bertolini et al., 1986; Bertolini, 1995; Guarini et al., 1999; Noera et al., 2001, 2002; Pinelli et al., 1989; Squadrito et al., 1999), or during prolonged respiratory arrest (Guarini et al., 1997). Melanocortins, including [Nle^4,D-Phe^7]α-MSH (NDP-α-MSH) and γ_1-MSH, are able, as well, to exert in rats a protective effect both in a model of transient myocardial ischemia followed by reperfusion, and in a model of permanent coronary artery occlusion. Such effect seems to be mediated by brain melanocortin MC_3 receptors (Bazzani et al., 2001, 2002; Guarini et al., 2002). Both the prevention of ventricular arrhythmias in transient myocardial ischemia, and the reduction of infarct size in permanent ischemia, may be due to the ability of melanocortins to inhibit the oxygen free radical discharge and to reduce the inflammatory response (Bazzani et al., 2001). It is well known, in fact, that melanocortin peptides have a peculiar, adrenal-independent, anti-inflammatory activity (for reviews, see Catania et al., 2000; Getting, 2002; Lipton and Catania, 1997; Wikberg et al., 2000).

It has been suggested that a non-melanocortin receptor belonging to a group of receptors for the molluscan peptide Phe-Met-Arg-Phe-NH_2 (FMRFamide), and for the mammalian analogues Tyr-Gly-Gly-Phe-Met-Arg-Phe-NH_2 (MERFamide) and Phe-Leu-Phe-Glu-Pro-Glu-Arg-Phe-NH_2 (neuropeptide FF; NPFFamide), may be involved in some of the cardiovascular effects of the γ-MSHs (Nijsen et al., 2000; Versteeg et al., 1998). This group of peptides shares with the melanocortin receptor agonists γ_1- and γ_2-MSH (but not with γ_3-MSH) the C-teminal Arg-Phe sequence and the anatomical location, including their receptors, within the brain (Fodor et al., 1996; Kivipelto et al., 1989, 1992; Majane et al., 1989; Nijsen et al., 2000; O'Donohue et al., 1984), and both groups provoke an increase in blood pressure and heart rate following systemic administration in normal rats (Allard et al., 1995; Mues et al., 1982; Nijsen et al., 2000; Thiemermann et al., 1991; Van Bergen et al.,1995, 1997; Versteeg et al., 1998). Also, the γ-MSH fragment γ_2-MSH-(6–12), which has no affinity for, and cannot activate any of the melanocortin receptor subtypes, contains the C-terminal Arg-Phe sequence and shows very potent pressor and cardioaccelerator effects (Nijsen et al., 2000; Van Bergen et al.,1995). On the contrary, melanocortins which lack the C-terminal Arg-Phe sequence, such as the melanocortin agonists ACTH-(1–24) and α-MSH, are devoid of pressor effects in normal rats; rather, ACTH-(1–24) decreases arterial pressure, but increases heart rate (Van Bergen et al., 1997; Versteeg et al., 1998). These findings suggest that the C-terminal Arg-Phe sequence, but not activation of melanocortin receptors, is essential for pressor and cardioaccelerator effects of melanocortins in normal animals.

The aim of the present study was to further examine which receptor and which structure are responsible for the protective effects of melanocortins in rats subjected to myocardial ischemia/reperfusion injury.

2. Methods

2.1. Animals and surgery

Adult Wistar rats of either sex, weighing 270–310 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 Committee on Animal Health and Care of Modena and Reggio Emilia University. The animals were acclimatized to our housing conditions for at least 1 week before use. Rats were anesthetized with urethane (1.25 g/kg i.p.; Fluka, Buchs, Switzerland) and fixed in the supine position on a heated operating platform, so to maintain rectal temperature at 37.5 °C. Under clean dissection, indwelling catheters were inserted into a common carotid artery and into a femoral vein. The arterial catheter was connected to a Statham P23 Db transducer for the recording of arterial pressure—and in some groups also of heart rate—on a polygraph (Mortara-Rangoni, Bologna, Italy). Mean arterial pressure was

automatically calculated by the poligraph; heart rate was automatically calculated by the same poligraph from the pulse wave. The venous catheter was used for drug administration; rats were given heparin i.v. (600 IU/kg; Sigma, St. Louis, MO, USA).

After cannulation of the trachea, the animals subjected to heart ischemia/reperfusion 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 within the normal range. The lead II electrocardiogram (ECG) was recorded by means of needle electrodes placed subcutaneously on the limbs. The chest was then opened by a left thoracotomy, the pericardium incised, and the heart gently exteriorized by pressure on the abdomen (Selye et al., 1960). A loose loop—5/0 braided silk suture attached to a 10 mm micropoint reverse cutting needle (Ethicon K-890)—was placed around the left anterior descending coronary artery, close to its origin. A polyethylene tube was threaded over the suture and the heart was replaced in the chest cavity with the ligature ends exteriorized, and any animal in which this procedure itself produced dysrhythmias or a sustained fall in mean arterial pressure to less than 60 mm Hg was discarded from the study at this point. After an equilibration period of 15 min, the ligature was tied. After a 5-min period of coronary occlusion, reperfusion was obtained by cutting the suture according to the device of Manning et al. (1989), and the animals were then monitored for a further 5 min, recording arterial pressure and occurrence of dysrhythmias and lethality.

In normal rats, arterial pressure and heart rate were monitored for 5 min.

2.2. Measurement of arrhythmias

The ECG was continuously monitored and recorded up to the fifth minute after reperfusion. Chart speed was set at 50 mm/s a few seconds before reperfusion so as to obtain a permanent high-speed recording of the changes in the ECG during early reperfusion. The ECG was retrospectively analyzed, in a blinded manner, for the incidence of ventricular tachycardia and ventricular fibrillation. All analyses were carried out in accordance with the Lambeth conventions (Walker et al., 1988). Ventricular tachycardia was defined as four or more consecutive premature beats of ventricular origin, and ventricular fibrillation was defined as a signal in which individual QRS deflections could no longer be distinguished from one another and for which the rate could not be determined.

2.3. Blood sampling, extraction of radical species and electron spin resonance (ESR) spectra determination

A technique modified from Tortolani et al. (1993) was employed in order to avoid the injection of the spin-trapping agent in vivo. Each animal had 3–4 ml of whole blood rapidly withdrawn via the arterial catheter into a syringe containing 2 ml of a 0.1 M solution of α-phenyl-*N*-*tert*-butylnitrone (PBN, Sigma) in isotonic saline. Each animal served for a single sample. The samples were immediately centrifuged ($1680 \times g$ for 10 min) and the plasma/PBN supernatant was added to 12 ml of 2:1 (v/v) chloroform/methanol for radical extraction. The chloroform layer was separated, dried under nitrogen flow, the resulting pellet was resuspended in 250 μl chloroform and the ESR spectrum was taken. ESR spectra were recorded at room temperature using a Bruker 300 ESR spectrometer (Bruker Spectrospin, Karlsruhe, Germany). Typical instrumental settings were as follows: microwave power, 20 mW; modulation amplitude, 0.1 mT; field width, 10 mT; microwave frequency, 9.14 GHz. The ESR peak height of the central absorption was measured, and expressed in arbitrary units (a.u.), as a direct function of adduct concentration. For statistical analysis, the values (a.u.) were normalised to a fixed sample volume of 1 ml of whole blood.

2.4. Drugs and treatments

γ_2-MSH, γ_2-MSH-(6–12), [D-Trp^8]γ_2-MSH and SHU 9119 were synthesized in our laboratory by solid phase chemistry, purified by HPLC and checked for proper molecular weight by mass spectroscopy, as previously reported (Grieco et al., 2000). ACTH-(1–24) and lidocaine hydrochloride were purchased from Sigma. All drugs were freshly dissolved in saline immediately before use, and injections were performed i.v. in a volume of 1 ml/kg.

Lidocaine, used as reference antiarrhythmic compound, was injected at the dose of 5 mg/kg; melanocortin peptides were administered at a dose equimolar (162 nmol/kg), or higher, to 0.48 mg/kg of ACTH-(1–24). Treatments with lidocaine and melanocortins were performed 2.5 min after coronary artery ligature (that is, 2.5 min before reperfusion). Pretreatment with SHU 9119 was performed 15 min before coronary occlusion. Doses and time were chosen on the basis of previous experiments performed in our laboratory using the same strain of rats and the same experimental procedure (Bazzani et al., 2001, 2002; Guarini et al., 2002). Control rats received equivolume amounts of saline. In normal rats, treatments were performed after an equilibration period of 10 min.

2.5. Statistics

The incidence of ventricular tachycardia, ventricular fibrillation or lethality were compared using Fisher's exact probability test. The free radical levels in arterial blood, mean arterial pressure and heart rate were analysed by means of one-way analysis of variance followed by Student–Newman–Keuls test. A value of $P < 0.05$ was considered significant.

3. Results

Myocardial reperfusion following a 5-min period of coronary occlusion, in saline-treated animals, caused within a few seconds a high incidence of ventricular tachycardia and ventricular fibrillation, and a high lethality within the 5 min of observation period (Table 1). As previously reported (Bazzani et al., 2001, 2002; Guarini et al., 2002), treatment with ACTH-(1–24), a non-selective agonist at all melanocortin receptors (Versteeg et al., 1998), at the dose of 0.480 mg/kg i.v. during coronary occlusion, almost completely prevented arrhythmias and strongly reduced lethality (Table 1). The anti-arrhythmic drug lidocaine (5 mg/kg i.v.) caused a significant reduction of ventricular fibrillation and lethality, but was less effective than ACTH-(1–24). γ_2-MSH, selective agonist at melanocortin MC_3 receptors (Versteeg et al., 1998), i.v. bolus injected at a dose (0.328 mg/kg) equimolar to the dose of ACTH-(1–24) (0.480 mg/kg, i.e., 162 nmol/kg), strongly reduced the incidence of ventricular tachycardia and ventricular fibrillation and lethality (Table 1). Also [D-Trp^8]γ_2-MSH, analogue peptide, highly selective agonist for the human melanocortin MC_3 receptors (Grieco et al., 2000), significantly prevented at the same dose (162 nmol/kg) the occurrence of ventricular tachycardia, ventricular fibrillation and death (Table 1). On the other hand, γ_2-MSH-(6–12), a fragment of γ_2-MSH which has no affinity for, and cannot activate any of the melanocortin receptors subtypes (Nijsen et al., 2000), administered at doses up to 324 nmol/kg during the ischemic period, failed to reduce the incidence of ventricular tachycardia, ventricular fibrillation and death (Table 1). The protective effect of ACTH-(1–24), γ_2-MSH and [D-Trp^8]γ_2-MSH on the incidence of ventricular tachycardia, ventricular fibrillation and death was significantly inhibited by pretreatment with the melanocortin MC_3/MC_4 receptor antagonist SHU 9119 (0.453 mg/kg i.v., equivalent to 320 nmol/kg: Hruby et al., 1995; Guarini et al., 2002) (Table 1).

As previously reported (Bazzani et al., 2001), the transient heart ischemia caused a large increase in the blood levels of free radicals 2 min after reperfusion (Fig. 1). The 2-min time period was chosen because, starting from 2.5 to 3 min after reperfusion, saline-treated control rats began to die. The feature of ESR spectra (not shown) hints to trapping of multiple radical species (lipid and proteins) arising from the radical burst. Treatment, during the ischemic period, with either γ_2-MSH or [D-Trp^8]γ_2-MSH (both at the dose of 162 nmol/kg i.v.) almost completely prevented the ischemia/reperfusion-induced increase in circulating free radicals (Fig. 1). On the other hand, γ_2-MSH-(6–12) (162 nmol/kg) did not affect the circulating levels of free radicals (Fig. 1). Myocardial reperfusion after the ischemic period was followed by an abrupt and marked fall in systemic arterial pressure in saline-treated rats, due to the occurrence of severe arrhythmias. The i.v. treatment during the ischemic period with either γ_2-MSH or [D-Trp^8]γ_2-MSH (162 nmol/kg), but not with γ_2-MSH-(6–12) (up to 324 nmol/kg), prevented such a fall (Fig. 2).

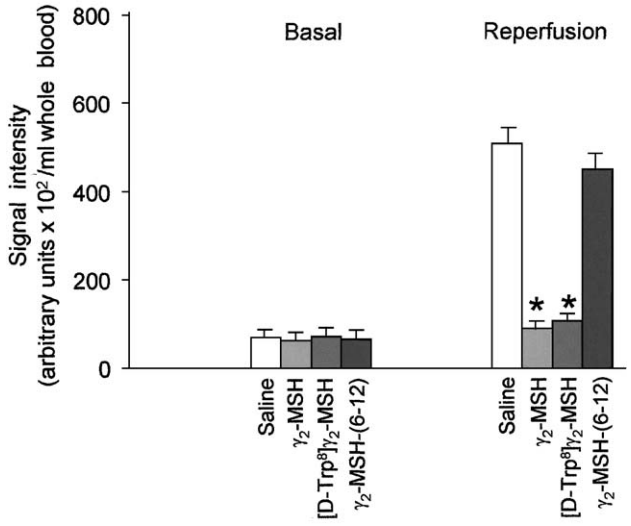

Fig. 1. Influence of γ_2-MSH, [D-Trp^8]γ_2-MSH and γ_2-MSH-(6–12) on PBN-adduct signal (ESR) intensity in rat blood, 2 min after myocardial reperfusion. Mean values ± S.E.M. for seven animals per group. Treatments with γ-MSH peptides (162 nmol/kg i.v.) or saline (1 ml/kg i.v.) were performed 2.5 min after left anterior descending coronary ligature (that is, 2.5 min before reperfusion). *$P<0.001$ versus saline-treated rats (Student–Newman–Keuls test).

Table 1
Influence of melanocortin peptides or lidocaine on the incidence of reperfusion-induced cardiac arrhythmias and lethality in anesthetized rats, during the 5 min following reperfusion

Pretreatment (mg/kg, i.v.)	Treatment (mg/kg, i.v.)	Incidence of:		
		Ventricular tachycardia	Ventricular fibrillation	Lethality
–	Saline	13/15	12/15	11/15
–	Lidocaine, 5	7/11	5/11[a]	3/11[a]
–	ACTH-(1–24), 0.480	3/12[d]	3/12[c]	2/12[c]
SHU 9119, 0.453	ACTH-(1–24), 0.480	8/9	7/9	6/9
–	γ_2-MSH, 0.328	4/12[c]	3/12[c]	3/12[b]
SHU 9119, 0.453	γ_2-MSH, 0.328	7/8	7/8	6/8
–	[D-Trp^8]γ_2-MSH, 0.328	5/14[c]	4/14[c]	4/14[b]
SHU 9119, 0.453	[D-Trp^8]γ_2-MSH, 0.328	7/9	7/9	7/9
–	γ_2-MSH-(6–12), 0.233	12/14	11/14	10/14
–	γ_2-MSH-(6–12), 0.466	8/10	7/10	7/10

Pretreatments were performed 15 min before coronary occlusion; treatments were performed 2.5 min after coronary occlusion (that is, 2.5 min before reperfusion). ACTH-(1–24), γ_2-MSH and [D-Trp^8]γ_2-MSH: 162 nmol/kg i.v.; γ_2-MSH-(6–12): 162 and 324 nmol/kg i.v. [a]$P<0.05$, [b]$P<0.025$, [c]$P<0.01$ and [d]$P<0.005$ versus saline-treated rats (Fisher's test).

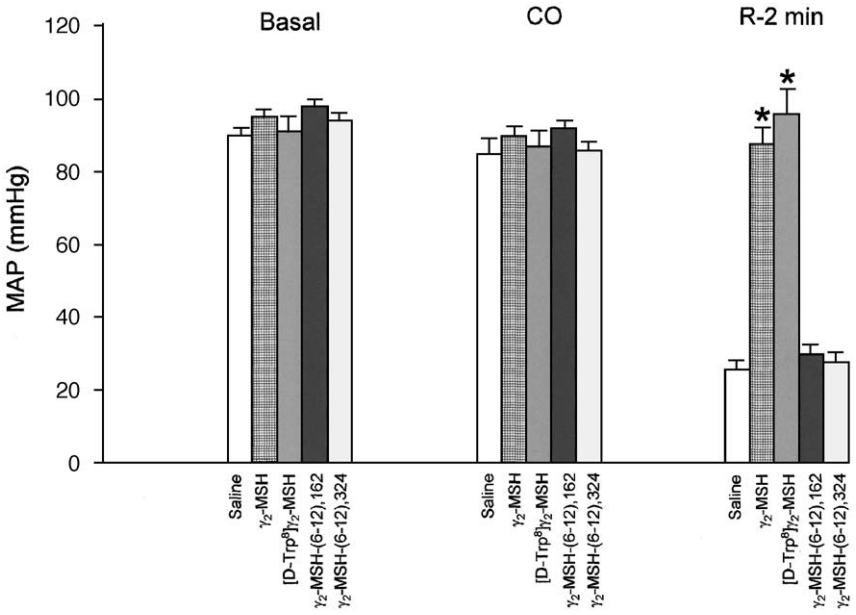

Fig. 2. Influence of γ_2-MSH, [D-Trp^8]γ_2-MSH and γ_2-MSH-(6–12) on the time-course of mean arterial pressure (MAP) in rats subjected to myocardial ischemia/reperfusion. Histogram heights indicate mean values ± S.E.M. for 10–15 animals per group. Treatments with γ-MSH peptides [162 nmol/kg i.v.; γ_2-MSH-(6–12) also at the dose of 324 nmol/kg i.v.] or saline (1 ml/kg i.v.) were performed 2.5 min after left anterior descending coronary artery ligature (that is, 2.5 min before reperfusion). CO: coronary artery occlusion; R-2 min: 2 min after reperfusion. *$P < 0.001$ versus the corresponding value of saline-treated rats (Student–Newman–Keuls test).

Finally, the i.v. injection in normal rats (i.e., rats not subjected to heart ischemia/reperfusion) of the melanocortin peptides able to prevent the occurrence of ventricular tachycardia, ventricular fibrillation and death, as well as the increase in free radical blood levels [ACTH-(1–24), γ_2-MSH and [D-Trp^8]γ_2-MSH], affected arterial blood pressure in different manner (Fig. 3). Just injected (within 15–40 s), γ_2-MSH and [D-Trp^8]γ_2-MSH (162 nmol/kg)

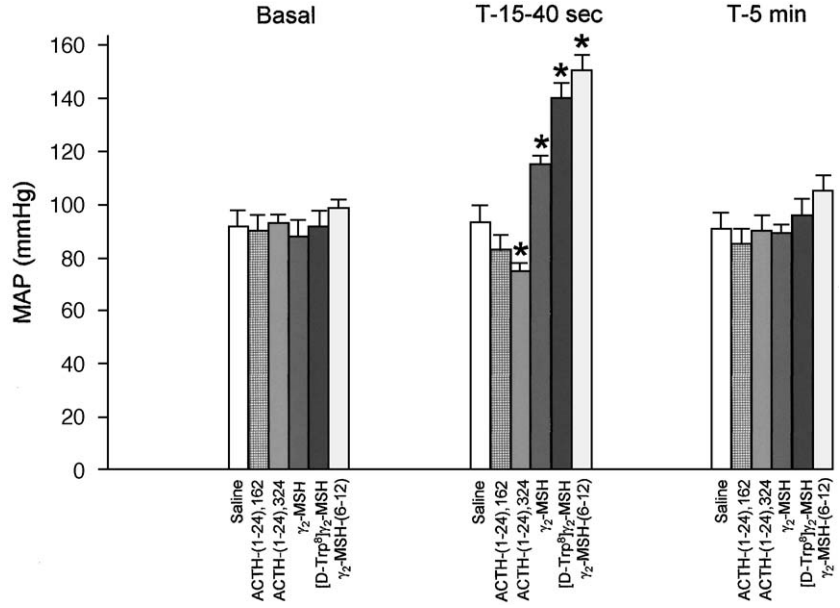

Fig. 3. Influence of ACTH-(1–24), γ_2-MSH, [D-Trp^8]γ_2-MSH and γ_2-MSH-(6–12) on the time-course of mean arterial pressure (MAP) in normal, anesthetized rats. Histogram heights indicate mean values ± S.E.M. for six to eight animals per group. All peptides were administered i.v. at the dose of 162 nmol/kg; ACTH-(1–24) was administered also at the dose of 324 nmol/kg. Saline: 1 ml/kg i.v.; T-15-40 sec: 15–40 s after treatment; T-5 min: 5 min after treatment. *$P < 0.05$, at least, versus the corresponding value of saline-treated rats (Student–Newman–Keuls test).

provoked a significant and transient increase in mean arterial pressure; ACTH-(1–24) caused a significant and transient decrease in mean arterial pressure only at a higher dose (324 nmol/kg). γ_2-MSH-(6–12) (162 nmol/kg i.v.), unable to prevent myocardial reperfusion injury, promptly (within 15–40 s) and transiently increased, in a significant manner, mean arterial pressure in normal rats (Fig. 3).

Heart rate of normal, non-ischemic rats was not significantly affected by any of the assayed peptides (not shown).

4. Discussion

In a condition of reperfusion following a short coronary artery occlusion, functional abnormalities of the reperfused myocardium may be lethal. These alterations are related to the formation of reactive oxygen species (Bolli, 1988; Bolli and Marban, 1999), which have the potential to directly damage cardiac myocytes and vascular cells, and may be involved in triggering the inflammatory cascade through the activation of NF-κB and consequent stimulated production of cytokines, including TNF-α (Altavilla et al., 2000; Chandrasekar and Freeman, 1997; Dhalla et al., 2000; Kupatt et al., 1999; Lefer and Granger, 2000). Indeed, NF-κB activation and TNF-α overproduction have been demonstrated in various models of experimental myocardial ischemia followed by reperfusion (Altavilla et al., 2000; Chandrasekar and Freeman, 1997; Kupatt et al., 1999). However, the severity of inflammatory response is related to the duration of ischemia (Frangogiannis et al., 2002; Lucchesi, 1990).

The present results confirm and extend our previous observations (Bazzani et al., 2001, 2002) that, in a condition of myocardial ischemia/reperfusion in the rat, melanocortin peptides are able to prevent the free radical discharge, the development of severe ventricular arrhythmias and the abrupt fall in systemic arterial pressure, with consequent increase in survival rate.

This protective effect of melanocortins may be due to indirect inhibition of oxygen free radical discharge—melanocortins have no direct scavenging activity (Guarini et al., 1996)—and to reduction of the inflammatory response. Melanocortins, in fact, have a peculiar anti-inflammatory activity (Catania et al., 2000; Getting, 2002; Lipton and Catania, 1997; Wikberg et al., 2000) linked to the stimulation of central and peripheral melanocortin receptors, and through the inhibition of NF-κB activation.

Melanocortins are known to reach the brain after systemic injection (Wilson, 1988), and a lot of experimental evidence points in this direction (for reviews, see Huang and Tatro, 2002; Guarini et al., 1999; Lipton and Catania, 1997; Versteeg et al., 1998). Previously, we suggested that the protective effect of melanocortins in myocardial ischemia/reperfusion-induced arrhythmias may be mediated by brain melanocortin MC_3 receptors (Bazzani et al., 2002; Guarini et al., 2002). In fact, such effect is obtained with a dose 10 times lower when the melanocortin peptide is administered intracerebroventricularly; moreover, it is obtained with the melanocortin γ_1-MSH, selective agonist at melanocortin MC_3 receptors. The present results, showing the efficacy of the selective melanocortin MC_3 agonists γ_2-MSH and [D-Trp^8]γ_2-MSH (Grieco et al., 2000; Versteeg et al., 1998), and the inhibition of such protective effect by pretreatment with the melanocortin MC_3/MC_4 receptor antagonist SHU 9119 (Hruby et al., 1995; Guarini et al., 2002), further support that melanocortin MC_3 receptors may be involved.

It has been suggested that γ_1-/γ_2-MSH and FMRFamide/MERFamide/NPFFamide could share an identical, non-melanocortin brain receptor involved in the cardiovascular stimulation of normal rat (Nijsen et al., 2000; Versteeg et al., 1998). In fact, these peptides share the C-terminal Arg-Phe sequence and the anatomical location, including their receptors, into the brain (Fodor et al., 1996; Kivipelto et al., 1989, 1992; Majane et al., 1989; Nijsen et al., 2000; O'Donohue et al., 1984). Moreover, it has been suggested that the C-terminal Arg-Phe sequence both in γ-MSHs and in FMRFamide-like peptides should be essential for cardiovascular actions (Nijsen et al., 2000). A key peptide for the identification of the receptor type involved in the cardiovascular and/or protective effects of γ_2-MSH and its analogues might be γ_2-MSH-(6–12), which contains the C-terminal Arg-Phe sequence and is the most potent γ-MSH fragment to induce pressor and cardioaccelerator effects (Van Bergen et al., 1995).

Now, the present data suggest that such non-melanocortin receptor of FMRFamide-like peptides is not involved in the protective effects of melanocortins against the myocardial ischemia/reperfusion-induced injury. In fact, γ_2-MSH-(6–12), which cannot activate any of the melanocortin receptor subtypes—but activates the receptor for FMRFamide-like peptides (Nijsen et al., 2000)—has no protective effect. Moreover, the C-terminal Arg-Phe structure is not relevant for the protective effect on the heart, because ACTH-(1–24), α-MSH and NDP-α-MSH, albeit lacking such sequence, are effective (present data; Bazzani et al., 2001, 2002; Guarini et al., 2002).

Finally, our data on cardiovascular effects of melanocortins in normal rats show that the pressor effect may be shared both by peptides which have protective effects in ischemic conditions [γ_2-MSH, [D-Tpr^8]γ_2-MSH] and by inactive peptides [γ_2-MSH-(6–12)]; moreover, some peptides which have protective effects [e.g., ACTH-(1–24), α-MSH, NDP-α-MSH: all three lack the C-terminal Arg-Phe sequence] have no pressor effects [rather, ACTH-(1–24) at high doses decreases arterial pressure] (present data; Van Bergen et al., 1997; Versteeg et al., 1998). It must be borne in mind that ACTH-(1–24)—in a dose-related manner—may decrease arterial pressure for a possible functional antagonism against γ-MSHs, and may increase heart rate for activation of the baroreceptor response and/or for the increase in sympathetic nerve activity (for review, see Van Bergen et al., 1997). It has been reported that γ-MSHs

provoke an increase in heart rate following systemic administration in normal rats (Nijsen et al., 2000; Van Bergen et al., 1995, 1997; Versteeg et al., 1998). In our experimental conditions, and at our doses, heart rate of non-ischemic rats was not significantly affected by any of the studied peptides. Moreover, melanocortins of the γ-MSH family may have two different effects on arterial pressure and heart rate: pressor and tachycardic effects are predominant in conscious rats and in rats under mild urethane anesthesia, whereas depressor effects, combined with a slight bradycardia, appear in rats under deep anesthesia, particularly with pentobarbital. Such depressor effects appear only when the sympathetic outflow is sufficiently suppressed (for review, see Versteeg et al., 1998).

In conclusion, the present data confirm and extend our previous findings that melanocortin peptides prevent the consequences of myocardial ischemia/reperfusion by activating melanocortin MC_3 receptors, likely located into the brain (Bazzani et al., 2002). Moreover, they further support the notion that, in normal rats, the cardiovascular effects of the melanocortin MC_3 agonists γ-MSHs are linked to other mechanisms, e.g., the activation of receptors for FMRFamide-like peptides. So in normal rats, cardiovascular effects of γ-MSHs and FMRFamide-like peptides seem to be dependent on the presence of the C-terminal Arg-Phe sequence, whereas this sequence is irrelevant for the protective effects of melanocortin MC_3 receptor stimulating γ-MSHs on ischemic/reperfused heart.

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