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Resuscitating effect of melanocortin peptides after prolonged respiratory arrest

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1 The resuscitating activity of melanocortin peptides (MSH-ACTH peptides) was tested in an experimental model of prolonged respiratory arrest.

2 Anaesthetized, endotracheally intubated rats subjected to a 5 min period of ventilation interruption, invariably died from cardiac arrest within 6–9 min of resumption of ventilation.

3 When resumption of ventilation was associated with the simultaneous intravenous (i.v.) injection of a melanocortin peptide (α-MSH or ACTH-(1–24)) (160 μg kg^{-1}) there was an almost immediate (within 1 min), impressive increase in cardiac output, heart rate, mean arterial pressure (+560% of the before-treatment value) and pulse pressure (+356% of the before-treatment value), with full recovery of electroencephalogram after 30–45 min. Blood gases and pH were normalized within 15–60 min after treatment, and all treated animals eventually recovered completely and survived indefinitely (= more than 15 days).

4 The same response was observed in adrenalectomized animals, as well as in animals pretreated with a β_1-adrenoceptor blocking agent (atenolol, 3 mg kg^{-1}, i.v.), or with an α_1-adrenoceptor blocking agent (prazosin, 0.1 mg kg^{-1}, i.v.), or with an adrenergic neurone blocking agent (guanethidine, 10 mg kg^{-1}, intraperitoneally).

5 An effect quite similar to that produced by melanocortins was obtained with ouabain (0.1 mg kg^{-1}, i.v.); the antioxidant drug, glutathione (75 mg kg^{-1}, i.v.) also produced 100% resuscitation, but the effect was slower in onset. On the other hand, adrenaline (0.005 mg kg^{-1}, i.v.) was able to resuscitate only 1 out of 8 rats and dobutamine (0.02 mg kg^{-1}, i.v.) resuscitated 4 out of 8 rats; moreover, the effect of both catecholamines was much slower in onset than that of melanocortins and the initial, impressive stimulation of cardiovascular function was absent.

6 These results show that melanocortin peptides have a resuscitating effect in a pre-terminal condition produced in rats by prolonged asphyxia. This effect seems primarily due to the restoration of cardiac function, not mediated by catecholamines. These data also suggest that these peptides may have potential therapeutic value in conditions of transient cardiac hypoxia and re-oxygenation such as occur in coronary artery disease.

Keywords: Respiratory arrest; hypoxia; melanocortin peptides; α-MSH; ACTH; resuscitation; inotropic agents

Introduction

The prompt availability of safe and simple resuscitating treatments capable of restoring cardiovascular and nerve function within a few minutes would be of major importance in the critical care of victims of traumatic accidents with massive blood losses and/or respiratory arrest. The severe and prolonged tissue hypoxia produced by rapid exsanguination or respiratory arrest is indeed the principal cause of death outside the hospital in victims of civilian or military trauma (Trunkey, 1983; Bellamy, 1984; Bellamy *et al*., 1986). The depth and duration of such a condition are major factors in subsequent in-hospital mortality rates. Measures taken in the field to limit the consequences of prolonged and severe tissue hypoxia are therefore of key importance in increasing survival (Baker, 1986).

We have previously shown that in an experimental condition of haemorrhagic shock in rats, produced by rapid removal of about 50% of the circulating blood and invariably causing the death of all untreated animals within 20–30 min of bleeding termination, the intravenous (i.v.) injection of melanocortin peptides (adrenocorticotrophin (ACTH), α-melanocyte stimulating hormone (α-MSH), other ACTH fragments: 1–24, 1–18, 1–17, 1–16, 4–10) in nanomolar amounts, dose-dependently restores arterial pressure, pulse amplitude and respiratory function, and produces a highly significant prolongation of the survival time (Bertolini *et al*., 1986a,b,c, 1989; Guarini *et al*., 1990; Bertolini, 1995). This effect is adrenal-independent (it is obtained also in adrenalectomized animals, and with melanocortin peptides practically devoid of corticotropic activity) (Bertolini *et al*., 1986a,c), and is associated with a massive increase in the volume of circulating blood, with consequent restoration of the blood flow (Guarini *et al*., 1987;1989), normalization of cardiac output and total peripheral resistances, gradual normalization of arterial and venous pH and base excess (BE), as well as of venous tension of O_2 (PO_2) and CO_2 (PCO_2) and venous oxygen saturation (Hb/O_2) and lactate (Bazzani *et al*., 1992), and with greatly reduced free radical levels in blood (Guarini *et al*., 1996). Although these peptides do not *per se* definitively cure haemorrhagic shock (all rats treated with the maximally active dose of 160 μg kg^{-1} die within 44$\pm$18 h) (Bertolini *et al*., 1989; Guarini *et al*., 1990), they nevertheless produce a rapid restoration of tissue perfusion in vital organs (Guarini *et al*., 1989), of degree and duration sufficient to extend considerably the time within which blood reinfusion can lead to an effective and definitive cure. In fact, while all rats reinfused with their own shed blood 15 min after haemorrhage die within 6.6$\pm$4.4 h, a substantial number of haemorrhage-shocked rats treated with ACTH-(1–24) shortly (5–10 min) after bleeding survive indefinitely even if blood reinfusion is performed 30, 60 or 120 min later (Bertolini *et al*., 1989; Guarini *et al*., 1990).

So far accumulated experimental data suggest that, in conditions of haemorrhagic shock, melanocortin peptides, which have negligible cardiovascular effects under normal conditions, except for γ-MSHs (Van Bergen *et al*., 1995) acti-

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vate, or restore, a complex vasomotor reflex that eventually leads to the mobilization of the peripherally-pooled residual blood; a reflex which is seemingly obtunded, in conditions of failure of the circulatory homeostasis, by the massive release of endogenous opioids (Bertolini *et al*., 1986a,b,c; Bertolini, 1995). This effect of melanocortins has been confirmed in dogs (Bertolini *et al*., 1986b) and in another model of hypovolaemic shock in rabbits (Ludbrook & Ventura, 1995), as well as in human conditions of haemorrhagic or cardiogenic shock (Bertolini *et al*., 1987; Pinelli *et al*., 1989; Noera *et al*., 1989, 1991).

An important question is whether such a remarkable property of melanocortin peptides is evident only in a condition of haemorrhagic shock or whether we are dealing with a more general resuscitating activity of these peptides, in whatever extreme, agonal condition. Accordingly, we have studied the effect of the two melanocortins most active in reversing haemorrhagic shock (ACTH-(1–24) and α-MSH) (Bertolini *et al*., 1986a,b,c; Bertolini *et al*., 1989) in an experimental condition of prolonged respiratory arrest, which proves fatal to saline-treated animals.

The results presented here show that indeed melanocortin peptides have a resuscitating effect also in such a pre-terminal condition. In an attempt to clarify the mechanisms involved, we in addition investigated the influence of α_1- and β_1-adrenoceptor blockade (by means of prazosin and atenolol, respectively), of sympathetic neurone blockade (by means of guanethidine), of vagal blockade (atropine), of catecholamines (adrenaline and dobutamine), of a cardiac glycoside (ouabain) and of an antioxidant drug (glutathione).

Methods

Animals and surgery

Adult female rats of a Wistar strain (Morini, S. Polo d'Enza, Reggio nell'Emilia, Italy) weighing 260–280 g, were used in strict accordance with the European Community regulations on the care and use of animals for scientific purpose (CEE Council 86/609, and Italian D.L. 27/01/92, no. 116), and all experiments were performed under general anaesthesia (ketamine (Farmaceutici Gellini, Aprilia, Italy) plus xylazine (Bayer, Milan, Italy), 100 + 2 mg kg^{-1} intraperitoneally (i.p.), plus a supplemental amount when necessary).

After heparine-treatment (heparin sodium 600 i u kg^{-1} in a tail vein; Prodotti Gianni, Milan, Italy) and clean dissection, a polyethylene catheter was inserted into a common carotid artery. Systemic arterial pressure and pulse pressure (PP) were recorded by means of a pressure transducer (P23 Db, Statham, Oxnard, CA, U.S.A.) coupled to a polygraph (Battaglia-Rangoni, Bologna, Italy); mean arterial pressure (MAP) and heart rate (HR) were automatically calculated and continuously displayed digitally by the polygraph. Cardiac output was measured as ascending aorta flow by placing, after thoracotomy in the third right intercostal space, an electromagnetic flow probe (inner diameter 2.5 mm; Nycotron, Drammen, Norway) around the ascending aorta immediately above the heart; signals from the flowmeter were recorded on the polygraph. Cardiac output was indexed for body weight and expressed as cardiac index (CI); total peripheral resistance index (TPRI) was calculated from the MAP and CI. For electroencephalogram (EEG) recording, the rats had been surgically prepared 1 week before the experiments by implanting, under ketamine plus xylazine anaesthesia, two stainless-steel screw electrodes above the frontal and parietal cortices; a reference electrode was placed in the thick bony area of the calvarium. EEG signals were recorded on the polygraph. The lead II electrocardiogram (ECG) was recorded by means of needle electrodes placed subcutaneously on the limbs. Rats were endotracheally intubated and ventilated with room air at a rate of 70 strokes min^{-1}, with a stroke volume of 20 ml kg^{-1} body wt. These ventilation parameters maintained blood gases and pH within the normal range (arterial blood: $P_{O_2} = 131.08 \pm 6.09$, $P_{CO_2} = 24.53 \pm 2.87$, $HCO_3^- = 17.70 \pm 1.99$, base excess (BE) = -4.94 ± 1.46, percentage haemoglobin saturation with O_2 (Hb/O_2) = 99.85 ± 0.40, pH = 7.43 ± 0.02; venous blood: $P_{O_2} = 45.01 \pm 4.01$, $P_{CO_2} = 36.50 \pm 1.05$, $HCO_3^- = 23.43 \pm 1.32$, BE = 0.83 ± 0.83, $SO_2 = 76.33 \pm 3.13$, pH = 7.40 ± 0.01 (mean ± s.e. mean; $n = 10$)). After a 10 min stabilization period following the surgical preparation, the ventilator was turned off to induce asphyxia for 5 min.

In a set of rats, bilateral adrenalectomy was performed by ventral approach under ethylether anaesthesia 7–8 days before the experiment. Sham surgery consisted of ethylether anaesthesia, ventral incision, entry of the peritoneal cavity and subsequent suture. Adrenalectomized animals were thereafter given saline instead of tap water to compensate for the absence of mineralcorticoid regulation of sodium. The adequacy of adrenalectomy was ascertained by estimation of the blood levels of corticosterone, measured by a radioimmunoassay method (Murphy, 1967), and by autoptical examination at the end of the experiment.

Drugs and treatments

After the 5 min period of respiratory arrest, the ventilator was turned back on, at which point ACTH-(1–24) (Ciba-Geigy, Basel, Switzerland) or α-MSH (Sigma, St. Louis, MO, U.S.A.) (160 μg kg^{-1}, in a volume of 1 ml kg^{-1}), or an equivolume of saline, were injected i.v. Rats were mechanically ventilated for 30 min following treatment (or until death); after this 30 min period, respiration was spontaneous. The dose of melanocortin peptides was chosen as the most effective in reversing haemorrhagic shock, on the basis of previous dose-response studies (Bertolini *et al*., 1989) performed in our laboratory in female rats of the same strain and age. Three groups of rats of equal size ($n = 6$) were pretreated, 20 min before respiratory arrest, with the β_1-adrenoceptor blocker atenolol (Imperial Chemical Industries, Milan, Italy) i.v. injected at a dose of 3 mg kg^{-1}, with the α_1-adrenoceptor blocker prazosin ·HCl (Sigma, St. Louis, MO, U.S.A.) i.v. injected at a dose of 100 μg kg^{-1}, or with the neurone-blocking drug guanethidine sulphate (Sigma, St. Louis, MO, U.S.A.) i.p. injected at a dose of 10 mg kg^{-1}. Drug doses were chosen as those certain to be effective in producing *in vivo* blockade of β_1- and α_1-adrenoceptors and sympathetic neurones, respectively (Varagić & Vojvodić, 1962; Berdeaux *et al*., 1977; van Meel *et al*., 1981).

In one set of experiments, rats were not prepared for cardiac output evaluation, but only subjected to the other minor surgical procedures (cannulation of a common carotid artery and implantation of epidural electrodes), and treated with ACTH-(1–24) ($n = 20$) or α-MSH ($n = 10$) after the 5 min period of respiratory arrest, simultaneously with ventilation resumption. Thirty minutes later they had their surgical wounds sutured, were allowed to recover from anaesthesia and then were maintained under standard conditions, one per cage, in the colony rooms where they were observed with no other treatment for a maximum of 15 days, to determine the survival time. At the end of this 15 day period, their locomotor behaviour, sensory-motor performance and learning ability were evaluated, in comparison with control rats of the same-strain, sex and age. Locomotor behaviour was assessed by means of the open field test (Benelli *et al*., 1988); sensory-motor performance was assessed by means of the test of Björklund *et al*., (1980); learning ability was assessed by means of the Morris water maze test (Morris, 1984).

In a subsequent set of experiments, 48 rats were randomly assigned to one of the following i.v. bolus treatments (in a volume of 1 ml kg^{-1}) at the end of the 5 min period of respiratory arrest: (1) ouabain·8 H_2O (Sigma, St. Louis, MO, U.S.A.), 0.1 mg kg^{-1}; (2) (−)-adrenaline-(+)-bitartrate (Sigma, St. Louis, MO, U.S.A.), 0.005 mg kg^{-1}; (3) dobutamine·HCl (Eli Lilly, Giessen, Germany), 0.02 mg kg^{-1}; (4) reduced glutathione (Sigma, St. Louis, MO, U.S.A.), 75 mg kg^{-1}; (5) atropine sulphate (Sigma, St. Louis, MO, U.S.A.),

1 mg kg^{-1} (at a rate of 0.1 ml 30 s^{-1}); (6) an equivolume of saline. The doses of ouabain, adrenaline and dobutamine which were used had previously been shown to have positive inotropic actions *in vivo* (Gregg & Fisher, 1963; Russel & Klaassen, 1973; Willerson *et al*., 1976) while glutathione, at the dose used, has been shown to have an antioxidant action *in vivo* (Ammon *et al*., 1986). The dose of atropine was chosen as that certain to be effective in producing vagal blockade (Brezenoff, 1973).

Statistics

Data are presented as means ± s.e. mean. Mean arterial blood pressure (MAP), pulse pressure (PP), HR, CI, TPRI, blood gases and pH values were analysed by means of ANOVA followed by Student-Newman-Keuls test; where appropriate, Student's *t* test was also used. The other parameters were analysed by means of Student's *t* test or ANOVA.

Results

During the period of asphyxia, EEG became isoelectric within 2 min of respiratory arrest, while ECG showed marked bradycardia, P wave inversion, partial atrio-ventricular block and S-T segment elevation (Figure 1). By the end of the 5 min period, MAP, PP, CI had decreased markedly (while TPRI had increased) (Table 1 and Figure 2), as had arterial and venous P_{O_2} (15.60 ± 2.16 and 12.01 ± 0.55, respectively), arterial and venous Hb/O_2 (15.97 ± 1.45 and 9.75 ± 0.81, respectively) and arterial and venous BE (−11.47 ± 0.78 and −7.11 ± 1.62, respectively). Arterial and venous pH had also decreased (6.99 ± 0.01 and 7.19 ± 0.01, respectively), whereas arterial and venous P_{CO_2} had increased (79.40 ± 2.76 and 53.11 ± 2.89, respectively) (mean ± s.e. mean; $n = 10$; in all cases: $P < 0.001$ versus the corresponding basal values; Student's *t* test). Only the HCO_3^- change was not significant, both in arterial and in venous blood.

The resumption of ventilation did not substantially modify the situation in saline-treated rats, and cardiac arrest within 6.27 ± 0.59 min (mean ± s.e. mean; $n = 30$) was the invariable final outcome. On the other hand, the i.v. injection of ACTH-(1–24) or of α-MSH produced in all rats an almost immediate (within 1 min) and impressive increase in cardiac output, HR, MAP (= +560% of the before-treatment value) and PP (= +356% of the before-treatment value) and a normalization of TPRI (Table 1 and Figure 2), with ECG normalization within 4 min after treatment and full recovery of EEG after 30–45 min (Figure 1). This was associated with a normalization of arterial and venous pH, P_{O_2}, P_{CO_2}, HCO_3^-, BE and Hb/O_2, measured 30, 60 and 120 min after treatment (data not shown; $P > 0.05$; ANOVA). Normalization was already complete 30 min after treatment, except for the BE values which took longer to be restored.

All rats allowed to recover from anaesthesia were still alive and apparently in normal health 15 days later. Their

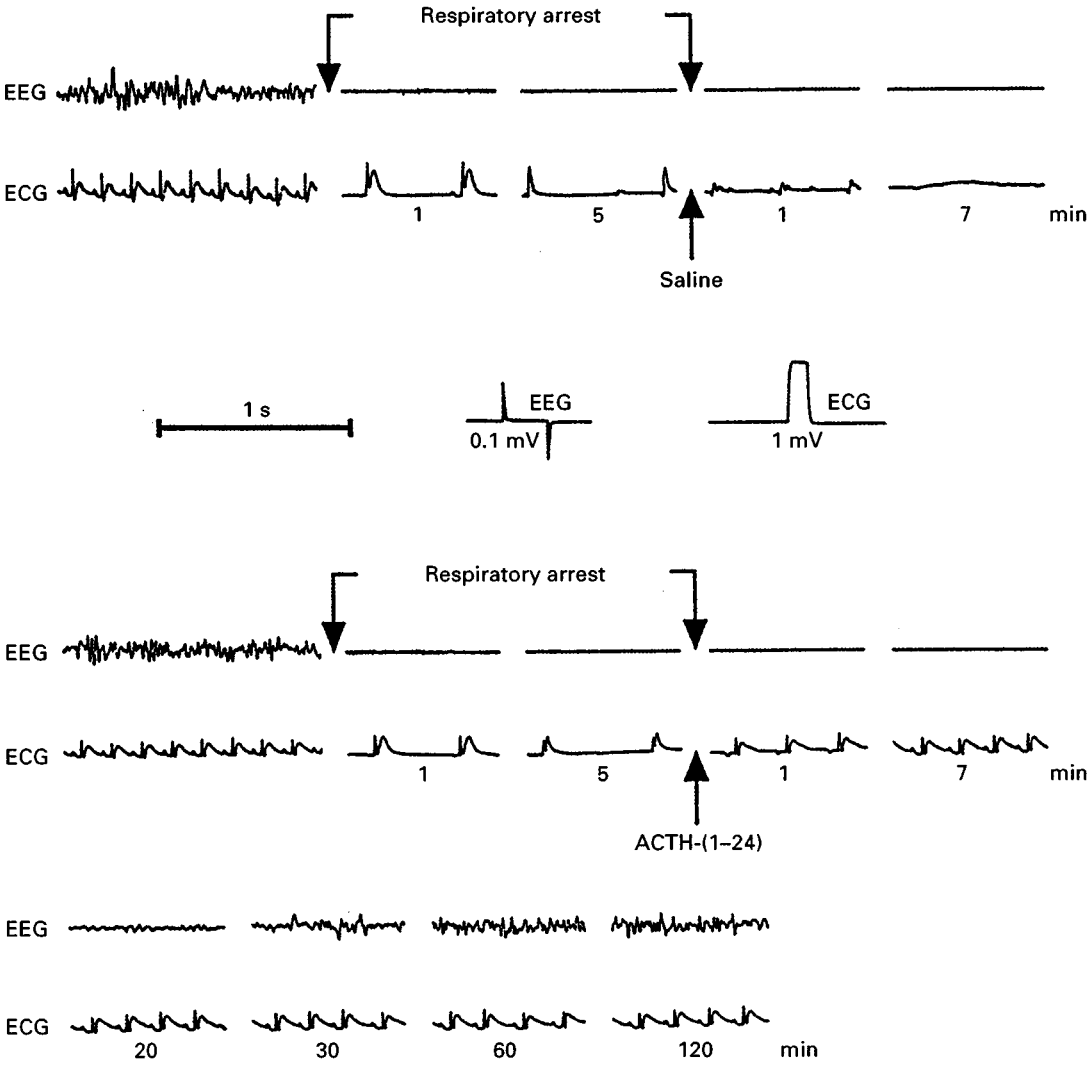

Figure 1 Representative recordings showing the effect of an i.v. injection of ACTH-(1–24) (160 μg kg^{-1}) or of saline (1 ml kg^{-1}) on EEG and ECG in rats subjected to a 5 min period of respiratory arrest.

Table 1 Effect of melanocortin peptides on cardiovascular parameters in rats subjected to a 5 min period of respiratory arrest

Treatment (i.v.)	*Parameter*	*Baseline*	*At the end of the 5 min respiratory arrest*	*At the following times (min) after treatment (with simultaneous ventilation resumption)* 1	5–8	30	120
Saline	MAP (mmHg)	83±4	25±2	27±3	0	0	0
1 ml kg^{-1}	PP (mmHg)	33±3	19±2	20±2	0	0	0
	HR (beats min^{-1})	375±12	85±4	85±5	0	0	0
	CI (ml min^{-1} 100 g^{-1} b.wt)	27.58±2.24	3.29±0.12	1.31±0.08	0	0	0
	TPRI (mmHg min^{-1} 100 g^{-1} b.wt ml^{-1})	3.03±0.18	7.71±0.39	21.15±0.71	0	0	0
α-MSH	MAP (mmHg)	90±4	25±2	165±6*	91±4	90±4	89±3
160 μg kg^{-1}	PP (mmHg)	33±3	18±2	82±3*	31±2	33±3	32±2
	HR (beats min^{-1})	360±11	90±3	330±12*	361±11	358±11	359±10
	CI (ml min^{-1} 100 g^{-1} b.wt)	28.01±2.25	2.99±0.11	18.71±1.75*	31.58±2.45	27.85±2.35	28.01±2.33
	TPRI (mmHg min^{-1} 100 g^{-1} b.wt ml^{-1})	3.28±0.16	8.42±0.41	8.87±0.37*	2.93±0.14	3.25±0.17	3.23±0.18
ACTH-(1-24)	MAP (mmHg)	80±4	26±2	160±5*	79±4	81±4	78±3
160 μg kg^{-1}	PP (mmHg)	30±2	18±1	75±3*	29±2	31±2	29±2
	HR (beats min^{-1})	370±11	86±4	345±11*	370±12	372±10	369±11
	CI (ml min^{-1} 100 g^{-1} b.wt)	27.41±2.38	3.18±0.11	17.55±1.73*	31.02±2.47	27.81±2.28	28.25±2.32
	TPRI (mmHg min^{-1} 100 g^{-1} b.wt ml^{-1})	2.95±0.15	8.21±0.42	9.16±0.41*	2.59±0.13	2.29±0.15	2.81±0.15

Data are presented as means±s.e.mean ($n=10$). *$P<0.001$ versus the corresponding value of saline-treated rats (Student-Newman-Keul's test). MAP - mean arterial blood pressure; PP - pulse pressure; HR-heart rate; CI - cardiac index; TPRI - total peripheral resistance index.

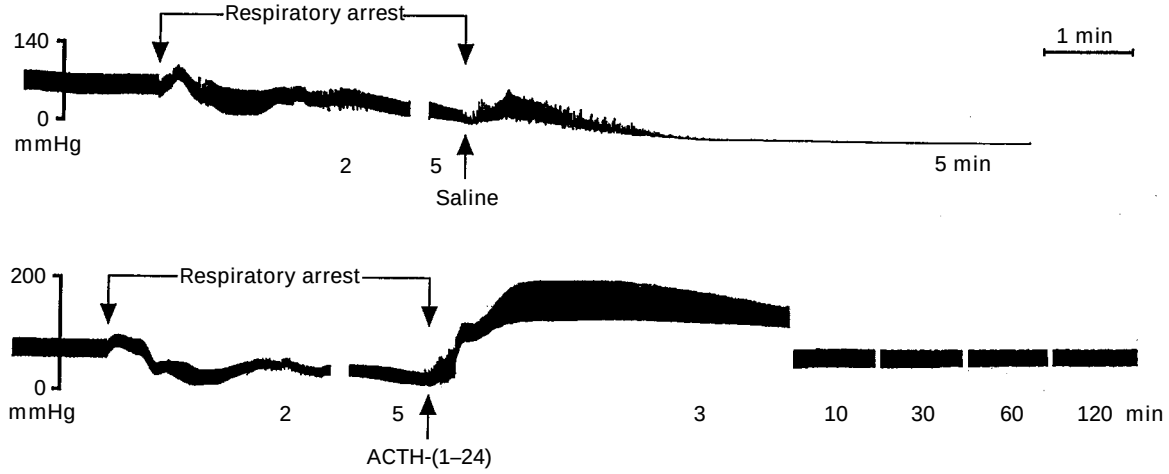

Figure 2 Representative recordings showing the effect of an i.v. injection of ACTH-(1–24) (160 μg kg^{-1}) or of saline (1 ml kg^{-1}) on arterial blood pressure in rats subjected to a 5 min period of respiratory arrest.

motor activity, sensory-motor performance and learning ability were not significantly different from those of control rats of the same strain, sex and age ($P>0.05$; ANOVA; data not shown).

In order to establish whether this effect of ACTH-(1–24) and α-MSH is due to their corticotropic activity (at the dose used in our experiments, α-MSH too may have strong corticotropic activity) (Desaulles *et al*., 1966), we repeated the experiment in adrenalectomized rats. Corticosterone serum levels were 0.010±0.002 μg ml^{-1} in adrenalectomized rats ($n=12$), and 0.195±0.009 μg ml^{-1} in sham-operated rats ($n=6$) (mean±s.e. mean; $P<0.001$, Student's t test). In these adrenalectomized animals the response to an i.v. injection of ACTH-(1–24) was the same as in non-adrenalectomized, sham-operated rats (Table 2).

Since the very first effect of ACTH-(1–24) and α-MSH in our experimental condition of prolonged respiratory arrest was a dramatic restoration of cardiovascular function, with impressive and prompt increase in cardiac output and PP (Table 1 and Figure 2), we also wanted to see whether such an effect may be due to the release of noradrenaline from sympathetic nerve terminals (ACTH-(1–24) increases the noradrenaline spillover rate and plasma noradrenaline concentration (Szabo *et al*., 1989)). However, not even this mechanism seems to be of relevance, for the effect of ACTH-(1–24) was not prevented by either blockade of β_1-adrenoceptors, by means of atenolol, or by the blockade of α_1-adrenoceptors, by means of prazosin, or by the functional blockade of peripheral sympathetic neurones, by means of guanethidine (Table 3).

Indeed, the administration of catecholamines (adrenaline and dobutamine) in pharmacological amounts was able to resuscitate some of the rats (1 out of 8 in the case of adrenaline, 4 out of 8 in the case of dobutamine), but the picture was quite different from that produced by α-MSH and ACTH-(1–24) (and much less impressive): the response was slower and the almost immediate and the massive increase in MAP and PP observed with α-MSH or ACTH (1–24) was absent (Table 4).

On the other hand, 100% resuscitation was obtained with the cardiac glycoside, ouabain, and with the antioxidant drug, glutathione; the effect of ouabain on MAP and PP and on HR was similar to that induced by melanocortin peptides, whereas that of glutathione was slower in onset and reached a maximum 5–8 min after injection (Table 4). However, ouabain was effective only at the dose of 0.1 mg kg^{-1} (Table 4), while it was

completely ineffective at the dose of 0.05 mg kg^{-1} (data not shown).

Finally, atropine-induced vagal blockade had no effect at all on recovery (Table 4).

Discussion

These results show that two melanocortin peptides, ACTH-(1–24) and α-MSH, have a resuscitating effect in an agonal

Table 2 Effect of bilateral adrenalectomy on the resuscitating effect of ACTH-(1–24) in rats subjected to a 5 min period of respiratory arrest

Animals	*Treatment* (i.v.)	*Parameter*	*Baseline*	*At the end of the 5 min respiratory arrest*	*At the following times (min) after treatment (with simultaneous ventilation resumption)* *1*	*5–8*	*30*	*120*
Adrenalectomized	Saline	MAP (mmHg)	73±4	24±2	26±2	0	0	0
	1 ml kg^{-1}	PP (mmHg)	35±3	18±1	20±2	0	0	0
		HR (beats min^{-1})	350±13	75±4	75±5	0	0	0
Sham-operated	ACTH-(1–24)	MAP (mmHg)	82±4	25±3	160±5*	81±4	78±3	79±4
	160 μg kg^{-1}	PP (mmHg)	33±3	18±2	83±4*	32±3	34±3	33±2
		HR (beats min^{-1})	362±11	80±4	320±12*	351±11	350±11	349±12
Adrenalectomized	ACTH-(1–24)	MAP (mmHg)	70±4	25±2	150±4*	69±3	71±3	71±4
	160 μg kg^{-1}	PP (mmHg)	34±2	17±1	78±3*	35±2	33±3	34±3
		HR (beats min^{-1})	355±12	76±4	305±13*	340±11	352±10	340±12

Data are presented as means±s.e.mean ($n=6$). *$P<0.001$ versus the corresponding value of saline-treated rats (Student-Newman-Keul's test). For key to abbreviations used see legend of Table 1.

Table 3 Effect of prazosin, atenolol and guanethidine on the resuscitating effect of ACTH-(1–24) in rats subjected to a 5 min period of respiratory arrest

Pretreatment (25 min before treatment)	*Treatment* (i.v.)	*Parameter*	*Baseline*	*At the end of the 5 min respiratory arrest*	*At the following times (min) after treatment (with simultaneous ventilation resumption)* *1–2*	*5–8*	*30*	*120*
Saline	ACTH-(1–24)	MAP (mmHg)	85±6	24±2	169±6	86±4	85±3	86±4
1 ml kg^{-1}, i.v.	160 μg kg^{-1}	PP (mmmHg)	35±4	18±2	85±4	35±3	34±3	35±3
		HR (beats min^{-1})	361±11	91±4	332±11	361±10	359±12	362±11
Prazosin	ACTH-(1–24)	MAP (mmHg)	89±7	23±2	160±6	90±4	91±5	90±4
0.1 mg kg^{-1}, i.v.	160 μg kg^{-1}	PP (mmHg)	34±4	16±1	79±4	35±4	34±3	34±4
		HR (beats min^{-1})	351±10	85±3	319±12	350±9	352±10	349±11
Atenolol	ACTH-(1–24)	MAP (mmHg)	81±6	25±2	166±5	80±3	82±4	81±4
3 mg kg^{-1}, i.v.	160 μg kg^{-1}	PP (mmHg)	33±2	18±2	78±3	32±2	33±2	32±2
		HR (beats min^{-1})	330±12	80±4	295±13	320±15	332±12	330±11
Guanethidine	ACTH-(1–24)	MAP (mmHg)	95±6	19±2	172±6	142±5*	90±4	60±5*
10 mg kg^{-1}, i.p.	160 μg kg^{-1}	PP (mmHg)	32±3	13±2	60±4*	33±2	32±3	31±2
		HR (beats min^{-1})	325±13	88±5	290±14	377±11	315±12	319±12

Data are presented as means±s.e.mean ($n=6$). *$P<0.05$ versus the corresponding values of saline pretreated rats (Student-Newman-Keul's test). For key to abbreviations used see legend of Table 1.

Table 4 Effect of ouabain, adrenaline, dobutamine, reduced glutathione and atropine on cardiovascular parameters in rats subjected to a 5 min period of respiratory arrest

Treatment (i.v.)	*Parameter*	*Baseline*	*At the end of the 5 min respiratory arrest*	*At the following times (min) after treatment (with simultaneous ventilation resumption)* *2*	*5–8*	*30*	*120*
Saline	MAP (mmHg)	91±5	22±2	16±2	0	0	0
1 ml kg^{-1}	PP (mmHg)	33±4	16±1	10±1	0	0	0
	HR (beats min^{-1})	365±15	88±6	78±4	0	0	0
Ouabain	MAP (mmHg)	99±5	21±2	128±5*	85±4	110±5	115±6
0.1 mg kg^{-1}	PP (mmHg)	30±3	16±1	60±5*	30±4	32±3	31±4
	HR (beats min^{-1})	305±15	71±6	270±10*	280±11	410±13	380±15
Adrenaline	MAP (mmHg)	80±5	21±2	39±24	81	70	59
0.005 mg kg^{-1}	PP (mmHg)	35±3	17±2	21±11	31	33	30
	HR (beats min^{-1})	330±12	76±5	149±46	321	328	379
Dobutamine	MAP (mmHg)	90±4	19±2	74±51	109±19	94±22	80±20
0.02 mg kg^{-1}	PP (mmHg)	30±2	15±1	27±16	27±5	22±3	22±3
	HR (beats min^{-1})	376±13	82±5	190±90	342±29	310±53	311±42
Glutathione	MAP (mmHg)	89±5	20±2	38±4	160±6	76±5	51±5
75 mg kg^{-1}	PP (mmHg)	31±3	15±1	21±2	62±4	21±3	25±3
	HR (beats min^{-1})	380±13	80±6	205±10	400±15	352±10	209±12
Atropine	MAP (mmHg)	95±6	21±2	17±2	0	0	0
1 mg kg^{-1}	PP (mmHg)	34±3	16±1	11±1	0	0	0
	HR (beats min^{-1})	352±14	83±6	75±5	0	0	0

Data are presented as means±s.e.mean ($n=8$). *$P<0.05$ versus the corresponding value of saline-treated rats (Student-Newman-Keul's test). From 5–8 min after treatment on, the values for adrenaline- and dobutamine-treated rats are those of surviving rats ($n=1$ and $n=4$, respectively). For key to abbreviations used see Table 1 legend.

condition produced in anaesthetized rats by a 5 min interruption of mechanical ventilation (these same peptides had been found to be the most effective ACTH fragments in reversing a condition of haemorrhagic shock in rats and dogs (Bertolini *et al*., 1986c, 1989)). Indeed, while not one of the saline-treated animals survived such a prolonged period of asphyxia and all died by cardiac arrest within 5–8 min of (and in spite of) ventilation resumption, all rats treated with either ACTH-(1–24) or α-MSH recovered completely and indefinitely.

The very first and primary effect of ACTH-(1–24) and α-MSH in such asphyxia-induced agonal condition is stimulation of cardiac activity, with about 4 fold increase of HR and 6 fold increase of CI. As a consequence (also because during the first few minutes TPRI remains high) there is a powerful increase of MAP and PP. About five minutes after treatment, HR and TPRI are restored to baseline, pre-asphyxia values, while CI is actually higher than in baseline conditions.

The functional recovery of the brain was slower and seemed to be the consequence of the restoration of blood flow and of normalization of haematochemical parameters. Indeed, ECG was already completely normal within 4 min after treatment, while full recovery of EEG occurred 30–45 min after treatment.

The effect of ACTH-(1–24) and α-MSH on heart activity in our experimental conditions did not seem to be mediated either by the release of adrenaline from the adrenal medulla or by the increased release of noradrenaline from sympathetic nerve terminals. Indeed, it was not prevented by either adrenalectomy or blockade of heart β_1- or α_1-adrenoceptors (the positive inotropic action of α_1-adrenoceptor stimulation in the heart is widely documented (for reviews see: Benfey, 1990; Fedida *et al*., 1993) and could be particularly relevant under hypoxic conditions, because hypoxia increases α_1-adrenoceptor density in cardiomyocytes (for a review see: Benfey, 1990)).

Moreover, the effects of ACTH-(1–24) and α-MSH were not mimicked by catecholamines or prevented by the sympathetic neurone blocker, guanethidine: only 1 out of 8 rats in the case of adrenaline, and 4 out of 8 rats in the case of dobutamine were resuscitated and, in addition, the cardiovascular response was completely different from that observed after α-MSH or ACTH-(1–24). Also pretreatment with guanethidine did not modify the response to ACTH-(1–24). On the other hand, the antioxidant drug glutathione and the cardiac glycoside ouabain were able to resuscitate all treated rats in our experimental model. However, while the effect of ouabain was rather similar to that of melanocortins, the effect of glutathione had a slower onset (we have previously shown that in the pre-terminal condition of haemorrhagic shock, in rats, the i.v. administration of melanocortins greatly reduces the blood concentration of oxygen-derived free radicals, that are greatly increased under such conditions (Guarini *et al*., 1996)). Finally, the possible pathogenetic role of increased vagal activity in our experimental condition can be excluded, because atropine was completely ineffective.

In conclusion, in a pre-terminal condition produced by prolonged respiratory arrest, melanocortin peptides, at nanomolar doses, far from toxic ones (Mandel *et al*., 1951), have an impressive resuscitating effect. It would seem, on the basis of the overall results obtained in our present research, that we are dealing with a direct effect of melanocortins on the heart. Such an effect might be mediated by specific receptors: although so far not one of the five classes of melanocortin receptors has been found to be expressed in the normal heart (Hol *et al*., 1995). However, this does not exclude the possibility either that they are expressed in the hypoxic heart or an as yet unidentified class of melanocortin receptor exists, as suggested by other experimental discrepancies (Adan *et al*., 1994).

In our opinion it is of special interest, both from a conceptual and possibly also from a practical point of view, that ACTH-(1–24) and α-MSH have such an impressive effect on the heart only in extreme hypoxic conditions (either caused by prolonged respiratory arrest or by massive haemorrhage) (Bertolini *et al*., 1986a,b,c, 1989; Guarini *et al*., 1990; Bertolini, 1995), while they have negligible cardiovascular effects under normal conditions (only γ-MSHs cause a dose-dependent increase in blood pressure and HR in normal rats (Van Bergen *et al*., 1995)). Were our present results to be confirmed in other conditions of heart failure, a quite new and unforeseen, and practically non-toxic (Mandel *et al*., 1951), class of positive inotropic agents would be available.

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