Review

Sheila Leone, Giorgio Noera and Alfio Bertolini*

Developments and new vistas in the field of melanocortins

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Abstract: Melanocortins play a fundamental role in several basic functions of the organism (sexual activity, feeding, inflammation and immune responses, pain sensitivity, response to stressful situations, motivation, attention, learning, and memory). Moreover, a large body of animal data, some of which were also confirmed in humans, unequivocally show that melanocortins also have impressive therapeutic effects in several pathological conditions that are the leading cause of mortality and disability worldwide (hemorrhagic, or anyway hypovolemic, shock; septic shock; respiratory arrest; cardiac arrest; ischemia- and ischemia/reperfusion-induced damage of the brain, heart, intestine, and other organs; traumatic injury of brain, spinal cord, and peripheral nerves; neuropathic pain; toxic neuropathies; gouty arthritis; etc.). Recent data obtained in animal models seem to moreover confirm previous hypotheses and preliminary data concerning the neurotrophic activity of melanocortins in neurodegenerative diseases, in particular Alzheimer's disease. Our aim was (i) to critically reconsider the established extrahormonal effects of melanocortins (on sexual activity, feeding, inflammation, tissue hypoperfusion, and traumatic damage of central and peripheral nervous system) at the light of recent findings, (ii) to review the most recent advancements, particularly on the effects of melanocortins in models of neurodegenerative diseases, (iii) to discuss the reasons that support the introduction into clinical practice of melanocortins as life-saving agents in shock conditions and that suggest to verify in clinical setting the impressive results steadily obtained with melanocortins in different animal models of tissue ischemia and ischemia/reperfusion, and finally, (iv) to mention the advisable developments, particularly in terms of selectivity of action and of effects.

Keywords: feeding; ischemic diseases; melanocortins; neurodegenerative diseases; sexual activity.

Introduction

Melanocortins comprise α, β, and γ melanocyte stimulating hormones (MSHs), adrenocorticotrophic hormone (ACTH), and several fragments containing at least the sequence His-Phe-Arg-Trp (=α-MSH 6-9). Moreover, they comprise several analogs that have been synthesized with the aim to improve receptor selectivity, potency, proteolytic stability, bioavailability, duration of action, biodistribution (particularly across the blood brain barrier, because the sites of action of melanocortins for their extrahormonal effects are mainly in the brain), and to obtain selective antagonists. The tetrapeptide His-Phe-Arg-Trp is the core sequence responsible for receptor recognition and activation, except for melanocortin 2 receptor (MC2R), which is exclusively activated by at least the 1–17 sequence of ACTH. For reviews, see Ref (1) and (2).

Natural melanocortins derive from the common precursor pro-opiomelanocortin (POMC), a protein of 31 kDa synthesized by cells widely distributed in the body: anterior and intermediate lobes of the pituitary, arcuate nucleus of the posterior hypothalamus, nucleus tractus solitarii of the medulla oblongata, adrenal medulla, gastrointestinal tract, pancreas, thyroid, testes, ovaries, skin, immunocytes (lymphocytes, macrophages) [for reviews, see Ref. (1, 2)]. The POMC sequence contains three main domains: the N-terminal pro-γ-MSH, the central ACTH, and the C-terminal β-lipotropin. Each domain contains one form of MSH: γ-MSH in the N-terminal domain, α-MSH as N-terminal 1–13 sequence of ACTH, and β-MSH in the β-lipotropin domain. This latter domain also includes the opioid peptide β-endorphin. The effects of melanocortins

***Corresponding author: Alfio Bertolini,** Department of Medicine, Section of Clinical Pharmacology, School of Medicine of the University of Modena and Reggio Emilia, Via Lanzi 30, 42020 Roncolo di Quattro Castella, Reggio Emilia, Italy, e-mail: bertolini.alfio@libero.it
Sheila Leone: Department of Pharmacy, University 'Gabriele d'Annunzio', Chieti, Italy
Giorgio Noera: Villa Maria Cecilia Hospital, Cardiac Surgery Unit, Cotignola (Ravenna), Italy; and Health R&S, Academic Spin Off, University of Bologna, Bologna, Italy

are mediated by five different receptors (MC1, MC2, MC3, MC4, MC5) that belong to the G-protein-coupled receptor family. They are functionally coupled in a stimulating fashion to adenylyl cyclase, and the effects of their activation are mediated primarily by a cAMP-dependent signaling pathway, with ensuing activation of protein kinase A and cAMP response element-binding (CREB) phosphorylation. Melanocortin signaling also involves intracellular free calcium elevation (following MC1 and MC3 stimulation), MAPK (especially ERK 1/2 activation), and Janus kinase/signal transducers and activators of transcription (JaK/STAT)(following MC5 stimulation) [for reviews, see Ref. (3, 4)]. The organism also produces two melanocortin antagonists/inverse agonists: the agouti protein (AgP), a 131 aa protein normally only expressed in the skin, which acts as high-affinity antagonist/inverse agonist at MC1, and antagonist at MC4 receptors (MC4Rs), and a structurally related 132 aa protein, the agouti-related protein (AgRP), normally expressed in a subset of arcuate nucleus neurons and in the adrenal gland, which acts as antagonist/inverse agonist at both MC3R and MC4R [for a review, see Ref. (1)].

The role of melanocortins in the physiology and pathology of mammals and of many other vertebrates is of increasingly recognized importance and wideness. In a broader sense, the POMC system, of which melanocortins and opioids constitute the most important moieties, with usually opposite effects (5, 6), plays a fundamental role not only inside the organism but also in its coping with the environment.

Besides the long-known endocrine functions of ACTH and MSH (corticotrophism, response to stress, pigmentation, and mimetism), melanocortins play an astonishingly wide range of roles in the physiology of the organism and have remarkable effects in several pathological conditions. Reproduction, fetal brain development, feeding, body homeostasis, inflammation and immune responses, pain sensitivity, drug addiction, motivation, attention, learning and memory, neurotrophism, and cardiovascular function are all influenced also by melanocortins, often importantly.

The discovery of these extrahormonal functions of melanocortins, of their effects in life-threatening situations, the synthesis of pharmaceutically suitable and highly potent and selective melanocortin agonists and antagonists, have opened unforeseen perspectives whose importance has probably not yet been fully perceived.

Here, we will briefly remind those aspects of the amazingly wide range of melanocortins' effects that are of special interest for pharmacology and therapy (together with some remarks on their discovery) and will review the recent developments, with special focus on those concerning the mechanism(s) of action and the therapeutic potential.

Reproduction

The induction of penile erections is one of the most characteristic and striking effects of melanocortins, together with excessive grooming and repeated acts of stretching and yawning [for a detailed description of this peculiar, complex, and bizarre behavioral syndrome, see Ref. (1)]. The site of action is in the brain. Testosterone plays a permissive role, and penile erections are not observed in castrated animals, in animals treated with cyproterone (antagonist at testosterone's receptor), and in animals with lesions in the brain areas accumulating testosterone, in particular the preoptic area (7). The presence of a receptive female is not needed, and several episodes of penile erection occur cyclically following a single administration: number and duration are dose and species dependent (8). In men, full penile erection may last up to several hours (9), but the mean duration (tip rigidity >80%) is about 40 min (10).

Also the sexual behavior of females is influenced: in female rats with a low level of sexual receptivity, melanocortins increase the lordotic response to male mounting, and estrogens play a permissive role (11). Melanocortins also facilitate the approach behavior based on sociosexual motivation in female rats (11).

The property of melanocortins to induce repeated episodes of penile erections also in the absence of sexual arousing stimuli was discovered almost half a century ago (7). In spite of the widespread therapeutic potential of such discovery and of the worldwide authoritativeness and diffusion of the publishing journal, it was almost completely ignored by the scientific community. Almost incredibly, such discovery was ignored also/even by the pharmaceutical industry (contrary to what happened in the case of sildenafil 27 years later).

Only many years after the paper of Bertolini et al., Hadley, while testing on himself the tanning efficacy of the synthetic α-MSH-analogue Melanotan-II (MT II), prepared in collaboration with Hruby and others, experienced a full, unrelenting erection lasting several hours: a funny account of this episode can be found in (9). He made a bibliographic research and discovered that his discovery had been discovered almost 30 years before: what he correctly quoted in his paper (12).

Both melanocortin-3 receptor (MC3R) and MC4R have been identified in the brain regions associated with activation of penile erections [particularly the paraventricular nucleus (PVN) of the hypothalamus], and the melanocortins more commonly used to induce penile erections [(α-MSH, tetracosactide (ACTH 1-24), MT-II, bremelanotide, etc.] activate both MC3R and MC4R. The first studies aimed

at defining what melanocortin receptor (MC) is involved in the induction of penile erections showed that the administration of HS014, an MC3/MC4 antagonist with a (relatively small) selectivity for MC4R, completely blocked the other behavioral effects of melanocortins (grooming, stretchings, yawnings), but only partially reduced penile erections, suggesting that MC3R is involved (13). Subsequent studies using MC4R knockout mice and MC4R antagonists much more selective than HS014 seemed on the contrary to provide evidence in support of MC4R as the one chiefly involved in the penile erection effect, albeit not excluding a role for MC3R. Further studies have actually shown that rats treated with a selective MC3R antagonist display significantly more erections after treatment with MT-II, and that the i.c.v. injection of a highly selective MC3R agonist fails to induce erections [for a review, see Ref. (14)].

Although the phosphodiesterase type 5 (PDE5) inhibitors (sildenafil and analogues) have provided a sufficiently safe and effective treatment of erectile dysfunction, and their commercial names are popularly reputed almost as synonymous of infallible sexual performance, a large proportion of men who complain of such disappointing and somewhat embarrassing inadequacy do not respond to PDE5 inhibitors or become less responsive or less satisfied as the duration of therapy increases (15). Also, men who are receiving organic nitrates cannot take PDE5 inhibitors because of nitrate interactions. In these subjects, melanocortin MC3/MC4 agonists have proven effective. In a clinical study enrolling 342 men with erectile dysfunction unresponsive to sildenafil, bremelanotide (20 mg as an intranasal spray) gave positive clinical results (responses to International Index of Erectile Function; weekly coitus episodes; mean intercourse satisfaction) in 33.5% patients, compared with 8.5% patients in the placebo group ($p=0.03$) (16).

Female sexual dysfunction, which incorporates various sexual disorders (hypoactive sexual desire disorder, sexual arousal disorder, orgasmic disorder, sexual pain), is also frequent: sexual arousal disorder affects about 30% women worldwide. Also in this case, bremelanotide (20 mg as an intranasal spray) significantly increased arousal score and intercourse satisfaction (17).

Food intake

Also in the presence of unrestricted food availability, wild animals (contrary to humans – particularly from 'civilized' countries – and to domestic animals) maintain a remarkably stable, species-distinctive, bodily shape. Such homeostasis is achieved through a complex, redundant system (where melanocortins play a fundamental role) that involves brain centers and multiple signals from other brain regions and from the periphery.

Peripheral signals reflect body fat stores and nutritional state and include nutrients and peptides produced by white adipose tissue, pancreas, and gut: leptin, insulin, ghrelin, glucagon-like peptide-1, peptide YY, and cholecystokinin. The hypothalamus integrates and processes such peripheral signals and induces appropriate changes in appetite and energy expenditure. The hypothalamic nuclei involved in the regulation of food intake and energy homeostasis include the arcuate nucleus, the PVN, the dorsomedial and ventromedial nuclei, the supraoptic nucleus, and the lateral hypothalamic area. These nuclei communicate with each other, and with other parts of the brain, via release of specific neuropeptides and through non-peptidergic neurotransmitters, including noradrenergic, dopaminergic, serotoninergic, histaminergic, and endocannabinoid signals. Extra-hypothalamic brain regions involved in the regulation of food intake and energy homeostasis, and which communicate with the hypothalamus, include the brainstem (which itself receives neural signals from the gut via the vagus nerve, and hormonal signals), the ventral tegmental area within the midbrain, and the nucleus accumbens in the striatum. The latter two brain regions are involved in the non-homeostatic regulation of feeding behavior, the so-called 'hedonic eating', a major responsible for overeating and obesity in humans.

The arcuate nucleus is pivotal to the integration and interpretation of signals of energy balance. Located at the base of the hypothalamus next to the median eminence with its incomplete blood-brain barrier, the arcuate nucleus is able to respond to circulating peripheral peptides such as leptin, insulin, and ghrelin. It contains two neuronal populations involved in feeding behavior and energy balance. One population produces the orexigenic peptides neuropeptide Y (NPY) and AgRP, the second population produces the POMC protein (which contains the sequences of the anorexigenic peptide α-MSH and of the orexigenic peptide β-endorphin) and a peptide encoded by cocaine and amphetamine regulated transcript (CART). The expression of NPY and AgRP mRNAs is increased by fasting, and the activity of these neurons is directly inhibited by leptin and insulin and stimulated by ghrelin. POMC/CART expressing neurons are also regulated by leptin and insulin, and their selective ablation causes hyperphagia and obesity. The expression of POMC and CART mRNA in the arcuate nucleus is decreased by fasting. Administration of leptin (the protein hormone

produced mainly by white adipose tissue that conveys to the brain information about the size of energy stores) directly into the arcuate nucleus inhibits feeding, and lesions of this nucleus attenuate the anorectic effect of leptin; moreover, leptin increases the expression of POMC mRNA in the arcuate nucleus, and the selective blockade of MC4Rs (that mediate the anorectic effect of melanocortins) significantly attenuates the feeding inhibition and the loss of body weight induced by leptin, indicating that leptin acts in the brain through the stimulation of melanocortinergic transmission upon hypothalamic centers involved in feeding control, and that melanocortin signaling occurs downstream to the leptin signaling. This is further supported by the observation that the anorectic effect of MC4 agonists is observed also in leptin-deficient mice (C57BL/6JLep^{ob}). The observation that the blockade of MC4Rs prevents the increase in mRNA uncoupling protein (UCP) expression induced by leptin in brown adipose tissue suggests that melanocortins are involved not only in the CNS but also in the periphery in mediating the effects of leptin [for a review, see Ref. (18)] through an α-MSH-dependent sympathetic outflow to adipose tissue ('browning of white adipocytes') (19). A schematic and far from complete picture of the peptidergic regulation of eating, especially centered on the role of melanocortins, is shown in Figure 1.

The first demonstration of the feeding inhibitory effect of melanocortins was obtained in the course of a systematic search for experimental arguments in support of the hypothesis (5, 6) that the POMC system exerts a balanced control on many body functions through the usually opposite effect of opioid and melanocortin peptides. Since it was known that opioids, through activation of brain kappa opiate (KOP) receptors, stimulate food intake, Poggioli, Vergoni et al. investigated the influence, if any, of melanocortins on such effect. Not only did they found that the i.c.v. injection of melanocortins (α-MSH and tetracosactide, at the doses of 4 or 10 μg/rat) abolished the feeding-stimulatory activity of KOP agonists (pentazocine, bremazocine, tifluadom), but markedly inhibited '*per se*' the spontaneous feeding of rats for many hours (20). Several years later, Fan et al. described '*…for the first time*..' the feeding-inhibitory effect of α-MSH (21), thus, albeit unknowingly, confirming Poggioli et al.'s data.

The place of melanocortins and, in a broader sense, of the POMC system in the feeding-regulatory system and in energy balance, and their role in eating disorders, are now well established and common knowledge, and plenty of reviews have been published on these subjects [for a list, see Ref. (1)]. The anorectic effect of melanocortins is extremely strong and is also observed in animals made hungry by a 24-h food deprivation or in the presence of stimuli known to cause vigorous feeding (insulin-induced hypoglycemia; stimulation of the noradrenergic or GABAergic systems) (22).

As mentioned above, the inhibitory effect of melanocortins on feeding is mediated mainly (probably exclusively) by central melanocortin MC4Rs. The targeted disruption of MC4Rs results in maturity-onset obesity, with hyperphagia, hyperinsulinemia, and hyperglycemia (23). The selective blockade of MC4Rs increases food intake in non-starved animals (100%–160% increase 4 h after treatment) and completely prevents the feeding-inhibitory effect of melanocortins. On the other hand, MC3Rs, which are largely presynaptically located, are thought to function as inhibitory autoreceptors on POMC neurons: it has been shown that the administration of a specific MC3 agonist stimulates feeding in mice (24). Melanocortins cause synapsin I phosphorylation in central vagal afferent endings and ERK 1/2 phosphorylation in the nucleus of the solitary tract: both mechanisms participate in the melanocortin-induced reduction of food intake (25).

Dominant mutations of the agouti locus that result in widespread ectopic expression of the AgP (high-affinity antagonist/inverse agonist at MC1 and antagonist at MC4Rs) cause the pleiotropic agouti obesity syndrome, characterized by hyperphagia, hyperinsulinemia, late-onset obesity, and inhibition of eumelanin pigment (obese yellow mice, lethal yellow mutant mice) (26); the i.c.v. administration of an MC4 agonist inhibits food intake in these animals. AgRP, which acts as antagonist/inverse agonist at both MC3R and MC4R, stimulates feeding, and its overexpression causes hyperphagia and obesity (27).

Besides playing a central role in the inhibition of the 'homeostatic', 'energy balance-regulated', 'hunger/satiety-controlled' eating, melanocortins, through the activation of MC4Rs located in the shell of the nucleus accumbens, also inhibit the 'non-homeostatic', 'hedonic' eating (28), whose driving force is gratification rather than energy deficit, and which is the major responsible for overeating and obesity (29, 30). To this aspect of the effect of melanocortins on feeding contributes, at least in part, the activation of oxytocin-containing pathways (31): the long-known inhibitory effect of oxytocin on feeding and drinking (32) also involves inhibition of reward circuits (33).

In humans, plenty of data have accumulated showing that either defects of the POMC gene or mutations in enzymes required for POMC processing or in the MC4R (more than 170 distinct mutations in the MC4R have been identified in humans) or MAPK signaling lead to a severe clinical phenotype with lack of satiety and early onset development of extreme hyperphagia and obesity

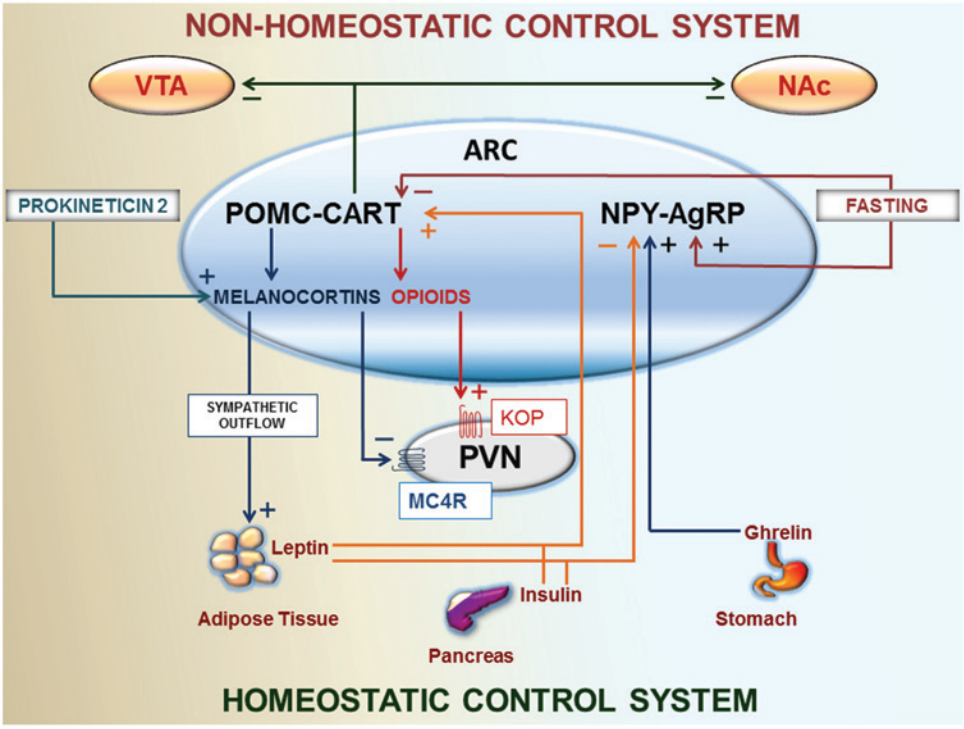

Figure 1: Place of melanocortins in the peptidergic regulation of food intake (either 'homeostatic', 'energy balance-regulated', or 'non-homeostatic', 'hedonic' eating) and energy expenditure and their relationships with other neuropeptides involved in body weight homeostasis.
Melanocortins (mainly N-acetylated α-MSHI), released by the POMC-synthesizing neurons of the arcuate nucleus of the hypothalamus (ARC) together with opioid peptides (mainly beta-endorphin), inhibit food intake through the activation of MC4Rs on neurons of the PVN and also increase the sympathetic outflow to brown adipose tissue, thus increasing energy expenditure. Opioid peptides, on the opposite, stimulate food intake through the activation of kappa opioid receptors (KOP) on neurons of the PVN. Another population of neurons of the ARC nucleus releases the NPY (the most potent orexigenic peptide known) and the AgRP, antagonist at MC4R. Leptin, produced mainly by the white adipose tissue and which conveys to the brain information about the size of energy stores, and insulin inhibit feeding through the stimulation of melanocortins production and the inhibition of NPY expression. Fasting and ghrelin (mainly produced by the stomach) stimulate the expression of NPY and inhibit that of melanocortins. The production of melanocortins is stimulated also by prokineticin 2. Melanocortins inhibit also the hedonic eating through an action on the shell of the nucleus accumbens (NAc) and the ventral tegmental area (VTA).

[for reviews, see Ref. (34)]. The MC4R mutations have been reported to occur in 4% of extremely obese children and are found to be the most common known monogenic cause of human obesity (35, 36).

Cachexia, or disease-related wasting, is common in cancer, chronic heart failure, chronic kidney disease, and severe infectious diseases; and is associated with anorexia, weakness, weight loss, muscle wasting, catabolism of protein and fat, increased resting energy expenditure, and reduction in bone mineral density. Also, severe stress and psycho-social stress lead to anorexia (whereas mild stress may cause overeating). Although caloric deficiency from anorexia is a common feature, cachexia is not prevented by increased caloric intake. There is increasing evidence to suggest that increased production of proinflammatory factors, including cytokines, chemokines, and eicosanoids, may play a crucial role in the pathogenesis of cachexia (37). Elevated serum levels of tumor necrosis factor-α (TNF-α), IL-1β, and IL-6 have been measured in patients with cardiac cachexia [see: (38)], and conversely, administration of proinflammatory cytokines, either peripherally or centrally, induces anorexia and weight loss (39). Data from an increasing number of studies support a critical role for the melanocortin system in the pathogenesis of cachexia (40). Mice with impaired central melanocortin signaling due to targeted deletion of the MC4R are resistant to the development of cachexia in models of lipopolysaccharide sepsis, cancer, and acute renal failure (40). Pharmacologic blockade of MC4R signaling with AgRP has been shown to attenuate cachexia in a murine model of cancer cachexia (41). Ghrelin, which potently inhibits melanocortin signaling by increasing

AgRP mRNA expression and hyperpolarizing POMC neurons, has been shown to attenuate the development of cachexia in rat models of cardiac heart failure (42). In rats, the administration of a selective MC4R antagonist reduces the immobilization stress-induced anorexia (43), and the administration of AgRP prevents the hypophagia induced by tumor growth (44). Collectively, these data support a direct role for the central melanocortin system in the pathogenesis of cachexia and suggest that blockade of central melanocortin signaling may represent an effective therapy for cachexia (the ameliorative effects of ghrelin in multiple types of cachexia, obtained in both animal and human studies, seem to be due to melanocortin modulation, in addition to its anti-catabolic and anti-inflammatory effects) (45).

Together with sudden hypoperfusion of organs of vital importance (heart and brain *in primis*), malignancies, and neurodegenerative diseases, obesity is one of the most important causes of disability, 'the greatest threat to public health in the developed world' (46). The first cause being overeating, coupled with inactivity. Overeating is stimulated by the abundance of food cues in the modern environment and the ready and easy availability (low physical effort and relatively low cost) of a wide variety of palatable, energy-rich food (high-fat and sucrose-sweetened diets) (29). The power of palatability, pleasantness, and variety to stimulate further food intake in satiated subjects is well known (the 'dessert phenomenon'). In addition, while proteins are the most satiating among macronutrients, fats appear to have a weak satiating capacity ('the fat paradox') (47). It is estimated that more than 1.5 billion individuals are overweight, and more than 400 million adults are obese (46). Most alarming is the ever mounting diffusion of overweight and obesity among children. Increased weight predisposes to, and can aggravate, many clinical conditions, including cardiovascular diseases, type II diabetes, restrictive lung disease, certain cancers, and infertility.

The key role of the melanocortin system in feeding behavior and energy homeostasis has raised much expectancy for a melanocortin-based, innovative approach to drug treatment of obesity and the interlinked metabolic syndrome, and, on the other hand, of cachexia. Many research groups and pharmaceutical companies are actively engaged in the synthesis of selective, pharmacokinetically suitable MC4R agonists and antagonists, also of non-peptide structure, for the treatment, respectively, of overweight and obesity, and of anorexia and cachexia. Several MC4R agonists are being developed and are entering phase I and II trials (48) [for an up-to-date and thorough survey, see Ref. (2)]. However, these conditions require long-term treatments, which may raise several problems, because the same subtype of MC receptors usually mediates different effects (e.g. on food intake, on sexual behavior, on the cardiovascular system, etc.) that in the long run may become upsetting, at the best, and because tachyphylaxis may develop. So, for example, it has been shown that the MC4R undergoes desensitization and internalization upon continuous agonist stimulation (49). In a phase II clinical study, the potent and selective MC4R agonist MK-0493 produced only a non-significant, 1.2-kg, placebo-subtracted weight reduction in obese subjects after 18 weeks of treatment (50). Similar results were obtained with the melanocortin fragment ACTH 4-10 (51). Following these disappointing results, it has been suggested that human obesity may be associated with melanocortin resistance (18). Another cause of concern is the possibility that the long-term administration of MC4 agonists may cause persistent signs of sympathetic nervous system activation with increased arterial pressure and heart rate. This has been the case of, for example, the MC4R agonist LY2112688 (52). This datum has been confirmed in a study on diet-induced obese non-human primates (rhesus macaques) (53). There would be an obvious concern in treating obese individuals with a high risk of hypertension and cardiovascular disease with a weight loss therapy that is exacerbating these same risks. However, this is not always the case. For example, in the same last quoted study (53), a highly selective novel MC4R agonist (BIM-22493), subcutaneously infused for 8 weeks, produced a 35% decrease in food intake and a 13.5% weight loss, with an associated improvement in glucose homeostasis and insulin sensitivity, and no increase in blood pressure or heart rate (yet, cardiovascular function was improved). A similar effect on body weight (13% reduction) and food intake (68% reduction) has been observed in diet-induced obese minipigs after 8 weeks of treatment with the selective MC4R peptide agonist MC4-NNI-0182. This compound, too, improved insulin sensitivity, as reflected by a 22% increase in peripheral glucose uptake (54).

Inflammation

Besides the well- and long-known effects of ACTH on inflammation and immune processes indirectly produced through activation of MC2Rs of the adrenal cortex with consequent synthesis and release of glucocorticoids, melanocortins exert a much more important, direct anti-inflammatory effect through activation of MC receptors (i) on immunocytes (macrophages, monocytes, neutrophils,

mast cells, dendritic cells, fibroblasts, microglia, astrocytes) (predominantly MC1R and MC3R, and also MC5R; the expression of MC1 receptors in these cells is stimulated by pro-inflammatory stimuli) and (ii) in discrete brain areas (predominantly MC4R) with ensuing activation of descending anti-inflammatory neuronal pathways [for reviews, see Ref. (2, 55)] (Figure 2).

The key mechanism responsible for the direct anti-inflammatory activity of melanocortins on immunocytes is the inhibition of the activation of the ubiquitary early transcription factor NF-κB (nuclear factor κB). In the cytoplasm, NF-κB is in a resting state, complexed to an inhibitory protein of the IκB family. A number of different stimuli, including infections, oxidative stress, endotoxins, proinflammatory cytokines, oxygen and nitrogen free radicals, and tissue hypoperfusion, cause the phosphorylation of IκB, which is followed by its degradation operated by proteasomes. The so free NF-κB translocates from the cytoplasm to the nucleus, where it binds the sequences of DNA encoding NF-κB responsive elements and triggers the transcription of hundreds of specific target genes and the production of several factors of the inflammatory response: cell adhesion molecules, inducible nitric oxide (NO) synthase (iNOS), chemokines, cytokines [in particular TNF-α], and cytokine receptors. Melanocortins inhibit the phosphorylation of IκB and hence the translocation of NF-κB into the nucleus and the consequent, above-mentioned, cascade of actors of the inflammatory response. Of particular interest is the selective effect of melanocortins on cytokines: through the action on IkB, melanocortins inhibit the production of pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, TNF-α), but on the other hand, they directly stimulate the production of anti-inflammatory cytokines (IL-8, IL-10). Moreover, melanocortins directly inhibit the antigen-stimulated lymphocyte proliferation [for reviews, see Ref. (2, 55)].

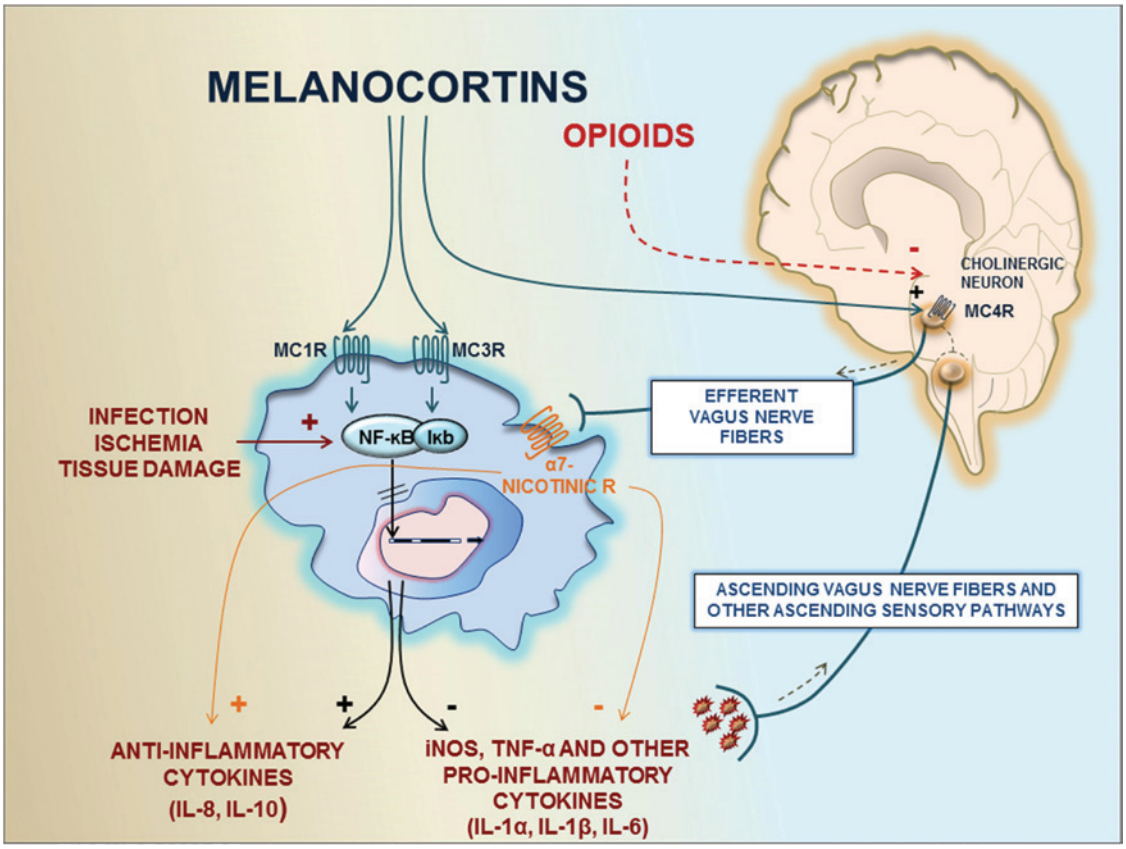

Figure 2: Mechanisms of the anti-inflammatory effect of elanocortins.
The non-adrenal-mediated antiinflammatory effect of pharmacological doses of melanocortins involves (i) the activation of MCRs (predominantly MC1R and MC3R) on immunocytes, with consequent inhibition of the translocation of the ubiquitary early transcription factor NF-kB into the nucleus and of the ensuing, NF-κB-dictated, overproduction of several factors of the inflammatory response; and (ii) the triggering of the 'inflammatory reflex' or 'brain cholinergic antiinflammatory pathway', which through the activation of α7-nicotinic receptors on immunocytes inhibits the overproduction and release of inflammatory cytokines while stimulating the production and release of antiinflammatory cytokines. (Opioid peptides, massively released during shock, on the contrary inhibit the 'inflammatory reflex'.)

Besides such effects on immunocytes, melanocortins also play an indirect anti-inflammatory effect by an action at the brainstem level, where they exert a tonic control (56) (MC3R and MC4R are present in the dorsal motor nucleus of the vagus and in the ventral division of the nucleus ambiguous) on the nervous reflex response called 'brain cholinergic anti-inflammatory pathway' or 'inflammatory reflex' [for a review, see Ref. (55)]. The brain plays an important modulatory role in inflammation. Inflammatory molecules produced in damaged tissues both (i) gain direct access to brain centers that do not have blood-brain barrier (dorsal vagal complex, area postrema, dorsal motor nucleus of the vagus, etc.) through the bloodstream, and (ii) activate afferent signals that are carried by ascending vagus nerve fibers and other pain and ascending sensory pathways (57). This neural inflammation-sensing pathway triggers in real time a nervous reflex response (even when the inflammatory agents are present in tissues in quantities not high enough to reach the brain through the bloodstream) with activation of efferent vagus fibers, acethylcholine release in organs of the reticuloendothelial system, and activation of α7-nicotinic receptors on immunocytes, which inhibits the release of TNF-α, IL-1β, and other inflammatory mediators of the early 'cytokine storm' (IL-6, IL-18) but not anti-inflammatory cytokines (IL-10) (58) (reflex 'brain cholinergic anti-inflammatory pathway') (59, 60).

Hypoperfusion/hypoxia

Whole-body hypoperfusion/hypoxia: shock, respiratory arrest, cardiac arrest

Melanocortins that are agonists at MC4R have an impressive resuscitating activity in several animal models of shock and in clinical conditions of severe hemorrhagic shock. Moreover, they have a likewise resuscitating effect in animal models of otherwise invariably fatal conditions of prolonged respiratory arrest [for reviews, see Ref. (1)].

The first accounts of the exciting results obtained in rats and dogs date back to about 30 years ago (61, 62). Straight after, such results were replicated in humans (63) and were the core topic of an international congress on 'Haemorrhagic shock: new vistas', whose proceedings were published in a dedicated issue of the journal Resuscitation (vol. 18, 1989). It seems to us not to be the case to describe here once again the details of such discovery and of the subsequent studies. In extreme concision, in subjects with massive blood losses, invariably leading to death in the absence of a prompt blood transfusion, the i.v. bolus injection of any melanocortin agonist at MC4Rs in pharmacological amounts (40–160 μg/kg) has '*per se*' a rapid resuscitating effect (restoration of blood pressure and respiration) without the need for prompt concurrent fluid infusion. Such treatment causes a temporary blockade of the processes that otherwise irreversibly lead to multiple organ failure and death, and produces a restoration of the blood flow in vital organs [due to the mobilization of the residual blood, that is peripherally pooled (capillary pooling and trapping of blood, 'capillary paralysis', that characterize the otherwise irreversible shock evolution)] of degree and duration (from 1–2 to several hours) sufficient to extend the time limit (the 'golden hour') (55) for a blood reinfusion to be definitively life-saving in such critical conditions. The obvious doubt that such an effect might be the consequence of a melanocortin-induced massive production and release of glucocorticoids by the adrenal glands was immediately cleared up by replicating the same results in adrenalectomized animals (62), or with melanocortins with no affinity for MC2Rs, and hence devoid of corticotropic activity (61). Melanocortins have also proven a resuscitating effect in experimental conditions of prolonged, otherwise invariably lethal, respiratory arrest (64).

The complex mechanisms underlying these impressive, life-saving effects of MC4R agonists have been by now definitively disclosed, also taking advantage of discoveries of other independent groups (e.g. discovery of the adrenal-independent anti-inflammatory activity of melanocortins by Lipton, Catania, and associates; discovery of the so-called vagus nerve-mediated 'inflammatory reflex' by Tracey and associates).

Briefly, the failure of the circulatory homeostasis, as it occurs in anyway-produced shock condition [hemorrhage, massive fluid losses (extensive burns, etc.), polytrauma, acute heart failure, etc.], and the consequent tissue hypoperfusion/hypoxia trigger, if not promptly corrected, a cascade of events that ultimately lead to multiple organ failure and death. Such events comprise the pooling of blood (or of the blood left in the body after ligature of the bleeding vessels, in the case of traumatic or surgical hemorrhage) in the capillaries of peripheral tissues and the consequent tissue hypoxia. Hypoxia triggers an unrestrained, disproportionate systemic inflammatory response ('systemic inflammatory response syndrome', SIRS) that starts within the first minutes and is the principal cause of the ensuing tissue damage. A key initiating role in such inflammatory cascade is played by the activation (mainly caused by oxygen and nitrogen free radicals, overproduced in response to tissue hypoperfusion) of

NF-κB and of mitogen-activated protein-kinases (MAPKs) (65, 66). Among the many factors of the inflammatory response whose production is triggered by NF-κB (see the previous section), TNF-α plays a fundamental role in the pathophysiology of shock: it inhibits noradrenaline release from sympathetic terminals, induces hyporeactivity of heart and vessels to noradrenaline, exerts a myocardial depressant effect, and further stimulates iNOS with massive overproduction of NO (which is one of the main responsibles for the hemodynamic decompensation and vascular hyporeactivity that occur after massive hemorrhage and in septic shock) [for a review, see Ref. (2)].

The effects of melanocortins on the inflammatory response have been described in the previous section. With regard to their indirect anti-inflammatory effect, exerted through the activation of the 'brain cholinergic anti-inflammatory pathway', it must be quoted that the melanocortin-induced reversal of shock is associated with a 4-fold increase in the efferent activity of vagus nerves (56) while being significantly reduced by bilateral vagotomy (43).

The functional antagonism exerted by melanocortins against opioid peptides [for review, see Ref. (1)] that are massively released in shock conditions (67–70) also plays a role of key importance: opioids inhibit sympathetic outflow and noradrenaline release from sympathetic terminals (70); contribute to peripheral pooling of blood, decreased venous return, and reduced cardiac output (71); and inhibit the above-described 'inflammatory reflex'.

These effects of melanocortins are of self-evident importance in clinical practice, because the prompt availability of safe and simple resuscitating treatments capable of restoring cardiovascular and nervous functions within a few minutes is of major importance in the critical care of victims of traumatic accidents with massive blood losses and/or respiratory arrest. Massive bleeding is a key issue in the treatment of trauma and surgery. It does account for more than 50% of all trauma-related deaths within the first 48 h following hospital admission, and it can significantly raise the mortality rate of any kind of surgery (72). The severe and prolonged tissue hypoxia produced by rapid exanguination or respiratory arrest is the principal cause of death outside the hospital in victims of civilian or military trauma (73, 74). The dept and duration of such a condition are major factors of 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 (75).

Since melanocortins have practically no acute toxicity in humans (76) and do not interfere with the action of the drugs routinely used in the treatment of shock (71), and owing to the fact that one of them [=tetracosactide (ACTH 1-24)] (i) had been marketed for many years for some other clinical conditions, with no or minimal side effects even after doses several times higher than those therapeutically used (76), and (ii) had been the most effective of the melanocortins used in our animal model of hemorrhagic shock, tetracosactide has been used as off-label drug in the clinical setting, in addition to the standard treatment, in several situations of hemorrhagic shock of various etiology [for a review, see Ref. (1, 2)]. In a study enrolling 32 subjects, all with type A aortic dissection complicated by aortic rupture and cardiac tamponade, and with clinical signs of shock, the survival at 1 month in patients assigned to the standard treatment (=surgery, volume restoration, inotropic drugs) was 47%, while in patients assigned to the same standard treatment plus an i.v. bolus injection of 10 mg tetracosactide at the moment of arrival into the casualty ward (20–40 min after the emergency call), the survival was 87% (77). Such 'spectacular results' (78) were replicated in a second clinical trial enrolling 112 subjects, and a phase III International clinical trial enrolling more than two hundred patients has been recently concluded successfully ('STOPSHOCK' trial, under the sponsorship of the Italian Ministry of Defence). A recent animal study (79) has confirmed the favorable effects of melanocortins also in a model of cardiac arrest induced in rats under general anesthesia by the i.v. injection of potassium chloride.

Hypoperfusion/hypoxia of single organs (heart, brain, intestine, kidney, testicles)

The results obtained in conditions of generalized tissue hypoxia prompted several groups to investigate whether melanocortin agonists may have protective effects also in conditions of organ-limited permanent ischemia or ischemia/reperfusion, where inflammatory processes also play a fundamental pathogenetic role both during ischemia and during reperfusion (66, 80).

Such studies have been performed either in different animal models [brain ischemia-reperfusion in dogs (81), global brain ischemia in rats (82), global cerebral ischemia followed by reperfusion in rats (83), incomplete permanent brain ischemia in rats (84, 85), transient cerebral ischemia in mice (86) and Mongolian gerbils (87), hearth ischemia-reperfusion injury in rats (88), hearth damage by permanent coronary occlusion in rats (89),

renal ischemic injury in mice and rats (90, 91), intestinal ischemia-reperfusion injury in mice and rats (92), and testicular ischemia-reperfusion injury in rats (93)] or in conditions of cerebral ischemia in humans (85, 94).

The results have shown without exception that the administration of a melanocortin peptide (α-MSH) or of melanocortin analogs [Melanotan I (MT-I), Semax (Met-Glu-His-Phe-Pro-Gly-Pro)] with agonist activity at MC4R and/or MC3R reduces to a highly significant degree the consequences of ischemia and ischemia-reperfusion either in terms of survival or of morphological and functional damage of the lesioned organ. In some cases, the therapeutic effect of melanocortins has been observed even when treatment started some hours after ischemia, this being of self-evident importance in medical practice.

Of particular interest, for their potential impact in clinical setting, should the results so far obtained in animals be confirmed in humans, are the data concerning heart and brain ischemia. In rats subjected to permanent ligature of the left anterior descending coronary artery, the volume of healthy myocardial tissue, measured 72 h after coronary occlusion and calculated as a percentage of the volume of the lateral wall of the left ventricle, was about three times higher in rats subcutaneously (s.c.) treated with MT-I (480 μg/kg every 12 h, starting 5 min after coronary ligature) than in saline-treated controls (89). Reperfusion of myocardium subjected to ischemia, although essential for salvage, is associated with severe ventricular arrhythmias, the main pathogenetic factors including a burst of oxygen free radical discharge, a Ca^{2+} overload in the intracellular space, the early activation of MAPKs and NF-κB, a rapid washout of extracellular protons, and the release of massive amounts of noradrenaline within the ischemic myocardium [for reviews, see Ref. (89, 95)]. In rats, the heart reperfusion following a 5-min ligature of the left anterior descending coronary artery produced, within a few seconds, severe ventricular arrhythmias with death of about 90% animals within the first 5 min. In such conditions, the i.v. injection of tetracosactide during the period of coronary occlusion (2.5 min before reperfusion) dose dependently reduced arrhythmias and lethality, with a 100% survival at the dose of 320 μg/kg (89), such effects being prevented by blockade of MC3Rs (88).

Likewise impressive results have been obtained in conditions of brain ischemia. In dogs subjected to brain ischemia/reperfusion, brainstem auditory evoked potentials were abolished within 10 min; treatment with α-MSH during ischemia almost doubled the recovery of such potentials with respect to saline-treated dogs (81). In rats, global cerebral ischemia followed by reperfusion reduced about by half the number of viable neurons in the CA1 pyramidal cell layer of the hippocampus; treatment with α-MSH at 30 min post-ischemia and then once daily for 4 days afforded an almost complete protection, '…suggesting a potential for α-MSH or mimetics thereof in the treatment of stroke or other neurodegenerative diseases' (83). In mongolian gerbils subjected to transient global brain ischemia followed by reperfusion, causing the death of almost 90% neurons of the hippocampus (CA1 region and dentate gyrus), treatment with the α-MSH analog ORG2766 reduced to about 40% the number of dead neurons (96). In a subsequent study, the same animal model caused delayed neuronal death in the CA1 subfield of the hippocampus, with subsequent impairment in spatial learning and memory; treatment with MT-I for 11 days after reperfusion dose-dependently reduced the histological damage and the learning and memory deficits , even when treatment was started up to 9 h after reperfusion (87). Similar protective effects have been obtained with the heptapeptidic melanocortin analog Semax. Besides improving survival and attenuating ischemia-induced neurological damage and learning and memory deficits, both in animal models and in human conditions of stroke, this melanocortin analog activated the transcription of neurotrophins [brain-derived neurotrophic factor (BDNF), TrkA, TrkB, TrkC, Nt-3, NGF] and their receptors in the cerebral cortex and in the hippocampus, and increased the proliferation of progenitor neuron cells in the subventricular zone (94). An increased neurogenesis in the dentate gyrus of the hippocampus, together with long-lasting improvement of learning and memory deficits, has been described also in mongolian gerbils subjected to transient global brain ischemia and treated with MT-I (97).

In bilateral renal ischemia in mice and rats, α-MSH significantly reduced renal damage even when treatment was delayed 6 h after ischemia (90).

Also, the severe injury to the small intestine produced in rats by ischemia-reperfusion was almost completely abolished by treatment with α-MSH (92): ischemia and reperfusion injury to the intestine are frequent and often extremely severe clinical conditions, with few effective therapeutic options.

Testicular torsion and the subsequent, surgically-induced, testicular detorsion cause an ischemia-reperfusion injury that can lead to infertility: in a rat model of testicular ischemia followed by reperfusion, the intraperitoneal treatment with MT-I reduced the histological damage and improved spermatogenesis; but in this case, a rather high dosage (340 μg/kg) and an unusually long treatment duration (once daily for 30 days, starting immediately after reperfusion) were required (98).

Traumatic and toxic damage of brain, spinal cord, and peripheral nerves

Much experimental evidence has accumulated showing that α-MSH , or other melanocortins containing the α-MSH sequence, or α-MSH fragments and fragment analogs containing at least the sequence 6–9 [His-Phe (or DPhe)-Arg-Trp](core message sequence), improve recovery in adult animals with traumatic, excitotoxic, or ischemic brain lesions, or with lesions of the spinal cord or of peripheral nerves.

In rats subjected to a severe lesion of the brain tissue (hemisection at the diencephalic level), the daily s.c. injection of α-MSH at the dose of 20 μg/rat for 2 weeks, starting on day 3 after lesion, hastened the recovery from sensorimotor deficits (99). The more potent and longer-acting analog MT-I , alike s.c. injected for 2 weeks starting on day 3 after lesion, but at the dose of 10 μg/rat every other day, was much more effective in restoring the sensorimotor functions, particularly coordinated limbs use and limb reflexes; moreover, it significantly reduced the apomorphine-elicited rotational behavior (100). The histological examination of the brains, removed on day 24 after lesion, showed in saline-treated rats large areas of acellular tissue at the level of the lesion, surrounded by numerous macrophages containing myelin debris, and a perivascular inflammatory picture characterized by marked histiocytosis. On the other hand, in rats treated with either α-MSH or MT-I, the areas of tissue destruction were significantly smaller; moreover, there was a reduced macrophage infiltration, no perivascular inflammatory reaction, and a large presence of hypertrophic astrocytes with elongated glial fibrillary antigen protein (GFAP)-positive processes at the point of lesion, more marked in rats treated with MT-I than in rats treated with α-MSH: it is worth quoting the view that a subset of astrocytes also functions as neural progenitor cells (101).

More recent data (102) have confirmed the protective effect of melanocortins in brain traumatisms. In a rat model of diffuse traumatic brain injury (the impact-acceleration model), the post-traumatic treatment every 12 h with MT-II, starting 3 or 6 h after injury, improved the functional recovery (sensorimotor orientation and limb use, evaluated at day 7, and learning and memory, evaluated at days 23–30 after injury) and was associated with a reduction in brain histologic damage (evaluated 1 or 2 days after the insult). Such effect occurred through the activation of MC4Rs and was associated with inhibition of the traumatic brain injury-induced up-regulation of NO synthesis, phosphorylation of extracellular signal-regulated kinases, c-jun N-terminal kinases and active caspase-3, and reduced expression and levels of TNF-α, BAX, IL-6, and High Mobility Group Box-1; while the levels of Bcl-2 (that suppresses apoptosis) and of the anti-inflammatory cytokine IL-10 were increased. In another model of traumatic brain injury ('controlled cortical impact' in mice), a single i.p. injection of the unusual C-terminal tripeptide α-MSH 11-13 (1 mg/kg 30 min after insult) reduced the volume of the lesion, the neuronal apoptosis, and the activation of microglia (103). In humans, a marked reduction in circulating α-MSH is found after acute brain injury of either traumatic or vascular origin, and patients with very low concentrations of α-MSH have a more unfavorable outcome (104).

Abundant data, especially obtained by Gispen and associates and Strand and associates, have accumulated showing that melanocortins have neurotrophic effects also on spinal cord axons and peripheral nerves through activation of MC4Rs (105) [for reviews, see (106, 107)]. The recovery from crush lesions of the sciatic nerve was accelerated by α-MSH, ACTH 4-10, ACTH 4-9, and its analog Org 2766 (108): melanocortins do not enhance the rate of outgrowth but rather increase the number of newly formed sprouts at the site of lesion. In a weight drop model of traumatic spinal cord injury in rats, α-MSH improved the recovery of lesioned animals (109). The α-MSH 4-9 analog Org 2766 has been extensively studied in several experimental and clinical lesions of peripheral nerves: significant protective effects have been obtained in diabetic neuropathy (110), cisplatin neuropathy (106, 111), and taxol neuropathy.

Neurodegenerative diseases

α-MSH has long been known as a trophic factor for the nervous tissue (112–116). In mammals, humans included, α-MSH is the most important trophic factor for fetal brain development (112, 117) and is present in high concentrations in the human fetal brain and pituitary. On the other hand, its concentration is significantly reduced in the brain of aging mammals (118) as well as in defined brain areas of patients with Alzheimer's disease (119). It stimulates the turnover of acetylcholine in the hippocampus and the central cholinergic neurotransmission [for reviews, see Ref. (112, 120), whereas aging is characteristically associated with progressive impairment of the brain cholinergic function (121, 122). The components of the behavioral syndrome induced by melanocortins (compulsive grooming, stretchings, yawnings, penile erections) are frequent in young and more and more rare in old animals (123). In

humans, yawning and stretching are already present at birth, and spontaneous penile erections occur very frequently in babies, whereas old people rarely yawn and even more rarely stretch or display full penile erections. Moreover, self-care and body cleaning (equivalent of grooming) are often neglected in the old. Melanocortins increase general arousal, improve the level of attention, and facilitate short-term memory (120): all of them are deteriorated in the elderly and most severely in Alzheimer's disease. In the fetal rat, injection of α-MSH antisera causes a decrease in fetal brain weight (124), whereas administration of α-MSH prenatally and neonatally improves the behavior of rats when juvenile and adult (125).

A role for melanocortins in neurodegenerative diseases, particularly Alzheimer's and Parkinson's diseases, has been repeatedly suggested and supported by many experimental data [for reviews, see Ref. (1, 2)]. Already 35 years ago, Landfield et al. (126) showed that the long-term treatment of middle-aged rats with the α-MSH-4-9 analog Org 2766 retarded both the morphological correlates of brain aging and the behavioral deficits that gradually develop during aging. α-MSH is reduced in the brain and cerebrospinal fluid of Alzheimer's patients, and α-MSH autoantibody levels correlate with cognitive dysfunction (119, 127). Moreover, α-MSH exhibits neuroprotective effects that may rescue neuronal degeneration: upregulation of CREB protein phosphorylation (128), induction of BDNF (129), and increased viability of hippocampal pyramidal cells (83, 130). Besides inducing BDNF expression, α-MSH induces MC4R expression and activates ERK and cFos in the rat brain (131). Most important, melanocortins have a quite peculiar, direct anti-inflammatory effect, and chronic inflammation plays a fundamental pathogenetic role in many neurodegenerative diseases: Alzheimer's disease, Parkinson's disease, multiple sclerosis, and amyotrophic lateral sclerosis (132–134). To our knowledge, the first, explicit hypothesis concerning α-MSH deficiency as a cause of Alzheimer's disease was formulated about 30 years ago (135). And the first, unequivocal (albeit indirect) experimental data allowing to suggest a beneficial effect of melanocortins in Alzheimer's disease were produced 20 years ago (136): in aged rats, the s.c. administration by constant infusion (10 nmol/rat/h) of an α-MSH-(4-9) analog (ebirtide), for 4 weeks, elevated the choline acetyltransferase activity in the septum (35% over controls), neocortex (79% over controls), and hippocampus (89% over controls). On the basis of these results, the authors suggested that ebirtide might prove beneficial in the therapy of brain degenerative disorders, especially Alzheimer's disease. Some years later, an '*in vitro*' study (cultured murine microglia cell line) (137) showed that α-MSH inhibits the accumulation of iNOS and TNF-α mRNAs triggered by the β amyloid proteins Aβ(1-42) and Aβ(25-35). The authors suggested that α-MSH might be used to moderate the brain response to amyloid beta deposition in Alzheimer's disease. Behavioral and histological data showing a protective effect of α-MSH in a mouse model of Alzheimer's disease have been quoted in Ref. (1): α-MSH had been loaded into polymeric nanoparticles specifically engineered with a glycopeptide and thus able to cross the blood-brain barrier and to target neurons and glial cells within the brain [for a review, see Ref. (138)]. More recently (139), it has been shown that in a mouse model of Alzheimer's disease of moderate severity (3xTg-AD) the daily i.p. treatment with MT-I for 18 weeks, starting at the age of 12 weeks, reduced both phosphorylation and levels of several biomarkers of Alzheimer's disease in cerebral cortex and hippocampus and improved spatial memory. These effects were prevented by the selective blockade of MC4Rs. In another contemporary study that used a different model of Alzheimer's disease (Tg CRND8 mice), a 4-week treatment with α-MSH failed to decrease the β-amiloid peptide load in the brain; however, it improved spatial memory by attenuating the loss of GABAergic neurons in the hippocampus, thus preserving the appropriate excitatory-inhibitory balance required for memory formation that is impaired in Alzheimer's disease (140). In another quite recent paper (141), the authors have confirmed their previous data (see above) using the same strain of mice and the same treatment and shown an increased number of mature, functionally integrated neurons in the dentate gyrus of the hippocampus of MT-I-treated animals.

A long-acting formulation of ACTH is used for the treatment of acute exacerbations of relapsing-remitting multiple sclerosis in alternative to high-dose methylprednisolone for patients who do not tolerate high doses of corticosteroids. According to a recent review (142), at least 55%–60% of patients who are steroid failures may respond to such ACTH formulation in different degrees. The effectiveness of ACTH in the treatment of acute exacerbations of multiple sclerosis in patients who have failed corticosteroids is explained by the different mechanisms of action of the melanocortin. ACTH is indeed a universal agonist for the five melanocortin receptors (MCs), all of which show a strong affinity for it. Thus, ACTH, besides stimulating the MC2Rs of the adrenal cortex, so mimicking the effect of corticosteroids, has high affinity and intrinsic activity also for MC1, MC3, MC4, and MC5 receptors. All these receptors (particularly MC1, MC3, and MC5 subtypes) are involved in the complex, direct, adrenal-independent, anti-inflammatory action of melanocortins (within the

brain, it is predominantly due to activation of MC4Rs): as reminded above, chronic, uncontrolled inflammatory processes in brain structures play a fundamental role in the pathophysiology of neurodegenerative diseases.

Many data are suggestive of a possible beneficial effect of melanocortins in Parkinson's disease, and it has been repeatedly hypothesized that melanocortin agonists may slow its progression (1 ,2, 143). The striatum is innervated by melanotropinergic fibers originating from α-MSH-containing cell bodies of the dorso-lateral hypothalamus (112, 144). α-MSH and several other melanocortin peptides increase the firing of nigral dopamine neurons and the striatal dopamine turnover (145, 146). Components of the behavioral syndrome induced by melanocortins (excessive grooming, crises of stretchings and yawnings) involve the activation of the nigrostriatal dopaminergic pathway (147), and it has long been known that parkinsonian patients rarely yawn and never stretch (148). In rats subjected to traumatic hemisection at the diencephalic level, with discontinuation of, among other pathways, the nigro-striatal connections, treatment with MT-I prevented the development of dopamine receptor supersensitivity in the striatum of the lesioned side and caused an increase in the dopamine receptors of the intact side (99).

Nephrophaties

The kidney is a target organ of the melanocortin system. MCs are abundantly expressed by multiple kidney parenchimal cells, including podocytes, mesangial cells, glomerular endothelial cells, and renal tubular cells.

ACTH was widely used in the 1950s–1960s as an effective therapy for childhood nephrotic syndrome, but has since been replaced by synthetic glucocorticoids, alkylating agents, and other immunosuppressants. Over the past two decades, ACTH has re-emerged as an effective therapy for nephrotic syndromes, particularly for patients who have failed the conventional immunosuppressive therapies, suggesting adrenal-independent mechanisms. Converging evidence unequivocally demonstrates that the melanocortin-based treatment has antiproteinuric, lipid-lowering, and renoprotective properties (149, 150)

Arthritic pathologies

Chondrocytes express functionally active MC1Rs and MC3Rs, which mediate the direct, adrenal-independent anti-inflammatory effects of melanocortins. It has been shown that in human C-20/A4 chondrocytes, both α-MSH and the selective MC3Rs agonist [DTRP(8)]-γ-MSH dose-dependently inhibit the TNF-α-induced release of pro-inflammatory cytokines (IL-1β, IL-6, IL-8); the expression of MMP1, MMP3, and MMP13 genes; the caspase-3/7 activation; and chondrocyte death; while increasing the production of the chondroprotective and anti-inflammatory cytokine IL-10 (151). The effects of [DTRP(8)]-γ-MSH, but not α-MSH, were abolished by the MC3R/MC4R antagonist SHU9119, indicating that both MC1Rs and MC3Rs are involved in such chondroprotective activity of melanocortins. These same melanocortin agonists reduced the injury caused by blunt impact on bovine articular cartilage explants (152).

Clinical evidence indicates that ACTH is highly effective in acute gout, its mechanism of action extending beyond stimulation of steroid release from the adrenal glands (153).

Miscellanea

Neuropathic pain

In the spinal cord of rats subjected to chronic constriction injury of the sciatic nerve with consequent neuropathic pain, both MC4R mRNA and POMC mRNA are upregulated (154). In these animals, the administration of an antagonist of MC3R and MC4R alleviates allodynia, whereas administration of MT II, agonist at the same receptors, has an opposite effect (155). These results suggest a possible therapeutic role for MC3R/MC4R antagonists in neuropathic pain.

West syndrome

ACTH and corticosteroids are still the gold standard treatment for this age-dependent (appearance at the 4th–8th month of life) epileptic encephalopathy (156) (Epub ahead of print), identified as a triad of infantile spasms (Salaam's tics), severe psychomotor retardation, and a specific EEG pattern (hypsarrhythmia). The use of vigabatrin has been gradually limited because it has been associated with the development of a serious constriction of the peripheral field of vision. It has been hypothesized that the long-known superiority of ACTH over corticosteroids could be due to the stronger inhibitory influence on corticotropin releasing hormone (CRH) secretion that far exceeds the inhibition

exerted by corticosteroids [it is widely accepted that CRH is implicated in causing West syndrome's spasms (157)].

Organ transplantation

Previous data (158) had shown that the protective effect of α-MSH against reperfusion injury and inflammatory reactions may increase the survival of transplanted organs. Heart grafts of rats treated with α-MSH showed an early expression of cytoskeleton proteins, transcription regulators and protease inhibitors, and a repression of immune and inflammatory mediators; later effects were down-regulation of oxidative stress response and up-regulation of phosphatidylinositol signaling system and glycolipidic metabolism. However, more recently, in a porcine experimental model, α-MSH did not reduce renal inflammation and did not improve short-term graft function following kidney transplantation (159).

Antimicrobial activity

α-MSH, its C-terminal and N-terminal sequences, and synthetic analogues have antimicrobial activity against both yeasts and bacteria (160) due to membrane disrupting properties and inhibition of DNA and protein synthesis. Most interestingly, in '*in vitro*' studies, the combination of α-MSH with oxacillin, gentamicin, rifampicin, tetracycline, and ciprofloxacin restored the bactericidal activity of these antibacterial drugs against a clinical isolate of *Staphylococcus aureus*, which was resistant to all of them (161).

Ebola hemorrhagic fever

Ebola virus infection causes in the host an unrestrained systemic inflammatory response that produces severe impairment of the vascular, coagulation, and immune systems, with ultimate tissue hypoperfusion, hemorrhages, multiple organ failure, and shock. A key initiating role in such inflammatory cascade is played by the activation of the ubiquitary early transcription factor NF-κB and of MAPKs. Owing to the mechanisms of action of the impressive resuscitating effect of melanocortins in shock conditions (see the previous section: 'Whole-body hypoperfusion/hypoxia'), tetracosactide has been used, together with other drugs, to cure the first Italian Ebola virus patient (Medical Bulletin of June 10th, 2015, National Institute for Infectious Diseases 'Lazzaro Spallanzani', Roma).

Expert opinion

Let us begin this section with an impression instead of an opinion. Our impression is that melanocortins are still viewed with a sort of skepticism in spite of (or just because of) their so unusually wide array of important effects, which are of potentially great impact in the treatment of some of the most frequent (and often among the leading causes of mortality and disability) pathological conditions: brain and heart ischemic insults, ischemia and ischemia/reperfusion of other organs, shock, bulimia, obesity, anorexia, cachexia, impotence in men and reduced sexual arousal and desire in women, etc.

For many years following the discovery of the curious effects of melanocortins on behavior (compulsive activity of grooming, stretching, and yawning; repeated episodes of unsolicited full penile erections) and on attention, learning, and memory, the skepticism about the meaning and importance of such effects was justified by the absence of proofs that melanocortins could affect the activity of brain centers. It was inconceivable that peptides synthesized outside the brain, in the pituitary, in extremely low amounts, could cross the blood-brain barrier, reach brain centers, and play a role in some brain functions. Such understandable skepticism was abated by the discovery in the 1980s that brain neurons synthesize and process a large protein (POMC) (appeared more than 700 million years ago, when the existence of living organisms on earth began to become more and more complicated) that contains the sequences of melanocortins, endorphins, and other peptides carrying extremely various, sometimes opposite, messages of key importance for the normal functioning of living organisms, for their reproduction, and for their survival in an extremely competitive, basically hostile, environment. The residual skepticism about the meaning of the presence of melanocortins in the brain was definitively removed by the discovery and cloning, more than 10 years later, of specific receptors that translate their messages.

On the other hand, skepticism still persists about the effects of melanocortins that are completely detached from their physiological functions: for example, the impressive effects in conditions of shock or organ-limited hypoperfusion/hypoxia, while melanocortins have no or negligible effects on the cardiovascular function under physiological conditions. And yet, findings obtained by independent groups have clarified the mechanisms of such effects (besides the one hypothesized at first, i.e. the functional antagonism between melanocortins and opioid peptides, which are released during massive hemorrhage, inhibits the sympathetic outflow, causes arteriolar vasodilation

and venodilation, and thus contributes to the failure of the microcirculation, which characterizes the condition of decompensated shock) (43): Lipton, Catania, and colleagues have shown that melanocortins have a peculiar, potent, adrenal-independent anti-inflammatory effect (see the previous section: 'Inflammation'); Tracey and associates have shown the existence of an 'inflammatory reflex' through which the brain inhibits the disproportionate inflammatory response to tissue hypoxia and to all other inflammatory stimuli by activating efferent vagus fibers; and finally, we have shown that the effect of melanocortins in shock and other conditions of tissue hypoperfusion/hypoxia is associated with a several-fold increase in vagal efferent activity while being largely prevented by bilateral vagotomy (56). So, the skepticism about the impressive effects of melanocortins in shock and any other condition of tissue hypoperfusion/hypoxia is no more justified. Particularly in shock, the dramatic effects of melanocortins have been repeatedly confirmed in humans using tetracosactide at the dose of 10 mg, bolus injected intravenously, and a phase III clinical study has been successfully completed recently. In view of that, tetracosactide should (must) be included in the first aid treatment of shock.

The capacity of MC4R and MC3R agonists to limit the morphological and functional consequences of ischemia and ischemia/reperfusion in all the organs so far studied (brain, heart, kidney, intestine, testicles) has been invariably confirmed by several independent groups in different animal models and, in the case of stroke, also in clinical setting. The impact of these pathological conditions on individual and public health is enormous in terms of mortality, invalidity, and costs. The results so far obtained with melanocortins, i.e. with drugs that for short-term treatments are practically devoid of toxicity and do not interfere with the other drugs currently employed in these cases, strongly demand that phase II and III studies be undertaken and, should the human studies confirm the animal results, that melanocortins be included in the treatment protocols of these diseases (in some countries, the melanocortin Semax is indeed already currently used). Particularly in the case of stroke, this also in view of the animal data showing that melanocortins (i) are effective in brain ischemia/reperfusion also when treatment starts several hours after the transient ischemic insult, and (ii) stimulate neurogenesis. The antipyretic activity of melanocortins could further help in protecting against stroke-induced neuronal damage (patients often present with, or develop, elevated body temperature after stroke, and this exacerbates outcomes). Also of great potential clinical interest are the results obtained in several studies showing that melanocortins improve recovery in traumatic brain and spinal cord injuries as well as in peripheral nerve lesions.

Moving to a much less serious situation, also the skepticism about the penile erection-inducing property of melanocortins lasted several years, and their use in erectile inadequacies is still enormously far from that of PDE5 inhibitors. And yet, melanocortins have proven effective in several cases of PDE5 failures and do not have the contraindications of PDE5 inhibitors. Sexual life seems to be increasingly unsatisfactory also for women: reduced sexual desire and sexual arousal, orgasmic problems, and sexual pain are increasingly frequent. Also in these cases, melanocortins produce significant improvements. In view of the limited effectiveness, of the contraindications, and of the disagreeable and rather problematic administration (in the case of prostaglandin E_1 or alprostadil), the other currently available therapies (essentially PDE-5 inhibitors and intracavernosal or intraurethral alprostadil) are far from fully satisfactory. Moreover, they do not affect sexual desire/libido. Thus, MC4 agonists active orally or as intranasal spray may represent a better option for the treatment of both female sexual interest/sexual arousal and orgasmic disorder (FSIAD) and male erectile dysfunction (162, 163).

Many attempts have been made in order to obtain molecules with selective agonist or antagonist activity at MC4Rs for the treatment of obesity or cachexia. In many cases, the results have been disappointing, clinical trials have been interrupted at phase II, and it has been hypothesized that obesity may be associated with melanocortin resistance. Moreover, in some cases, the long-term administration of MC4 agonists has produced persistent signs of sympathetic activation (melanocortins increase sympathetic outflow and noradrenaline release from sympathetic terminals) with increased heart rate and arterial pressure. These side effects are of special concern in obese subjects, who are at risk of cardiovascular complications. However, recent studies have produced more encouraging results: new MC4 agonists, administered for 2 months, have been well-tolerated, with no side effects at the cardiovascular level, persistent reduction of food intake, highly significant reduction of body weight, stimulated energy expenditure, and increased insulin sensitivity. As concerns cachexia, the data so far obtained in animal models suggest that the blockade of MC4Rs may represent an effective approach. Also, the effectiveness of ghrelin in several models of anorexia is likely the consequence of the ghrelin-induced potent inhibition of melanocortin signaling.

Both the rational bases and the (so far scarce) experimental results showing that melanocortins limit

the behavioral and histological deterioration in animal models of Alzheimer's disease, actually stimulating the hippocampal neurogenesis, request the replication of these studies in other models in different animal species. A great number of drugs are under development, either as patented candidates or as compounds already in clinical trials, for the treatment of Alzheimer's disease (164). Among them, also small peptides (of 8-9 aa) have shown neuroprotective activity in both '*in vitro*' and '*in vivo*' studies conducted in relevant Alzheimer's disease models (165). However, should the data obtained with melanocortins be confirmed, human studies could be advisable. The biggest issue in the case of an employment in clinical setting would be the duration of the treatment (several/many years), with probably unbearable incidence of side effects (anorexia, sexual arousal [already often very disturbing in these patients], hypertension, and tachycardia): cycles of treatment might be envisaged in this case.

Similarly, the employment of melanocortins in Parkinson's disease, although suggested by experimental data, seems problematic. In this case, the treatment should last uninterrupted for several years, with highly probable disturbing consequences (especially sexual arousal, already induced by the standard dopaminomimetic treatment, could become almost unmanageable).

The beneficial effect of ACTH in multiple sclerosis is, on the other hand, fairly well established. In this case, the problem of side effects is lower because the treatment duration is limited to the acute exacerbations.

Outlook

The site of action of melanocortins for their extra-adrenal effects is mainly in the brain, and indeed, most of such effects have been discovered following the injection of ACTH 1-39 (the natural ACTH), ACTH 1-24 (tetracosactide), or α-MSH (=ACTH 1-13) directly into a cerebral ventricle or into the cisterna magna (stretchings, yawnings, excessive grooming, penile erections) (166). This because natural melanocortins (ACTH 1-39, α-MSH) or their fragments (like ACTH 1-24) are peptides and, under normal conditions, only to an extremely limited degree can cross the blood-brain barrier. Such limitation concerning bioavailability and biodistribution into the CNS does not apply in the case of shock or brain ischemia and of other extremely severe conditions (sepsis, etc.), where the blood-brain barrier is severely compromised. For the other indications, great efforts have been made, and are being made, in order to obtain melanocortin analogues with much improved bioavailability, potency, receptor selectivity, agonist, or antagonist activity. As concerns bioavailability and potency, the results have been impressive, and several, highly potent melanocortin analogues are available even for oral administration [for a review, see Ref. (2)]. Of much conceptual and practical interest are the studies aimed at incorporating drugs of peptide structure (or anyway unable to enter into the brain, such as anti-tumor agents) into nanocarriers as polymeric nanoparticles, liposomes, nanoassembly, and dendrimers able to cross the blood-brain barrier and to release in the brain the transported therapeutic agent (138). As concerns receptor selectivity and separation of agonist and antagonist activities, on the other hand, the results have not yet been fully satisfactory, and further efforts will be made in the next future in this direction. Another problem is represented by the fact that the activation of a single type of MC receptor usually produces multiple effects. This can compromise the possibility of a clinical use of melanocortins in chronic diseases (typically Alzheimer's and Parkinson's diseases) because of the unavoidable suite of increasingly unmanageable unwanted effects (anorexia and progressive loss of body weight; repeated, in the end disturbing, stretchings, yawnings, penile erections, and/or sexual arousal; and hypertension and tachycardia). Such side effects are unacceptable in old patients already often suffering from anorexia, cardiovascular diseases, and severe behavioral problems. So, in the next years, a formidable task should be the characterization of subtypes of MC receptors, mediating single effects, and the synthesis of agonists (and antagonists) selective for such receptor subtypes.

Quite different is the case of acute clinical conditions, which require short-term treatments (days, few weeks, or even a single administration): shock (whatever caused), respiratory arrest, cardiac arrest; ischemia and ischemia/reperfusion of brain, heart, intestine, kidney, etc.; and traumatic or toxic insults to brain, spinal cord, and peripheral nerves. In these cases, there is no/weak possibility of side effects due to the brevity of the treatment, or the side effects would be of no relevance in view of the severity of the pathological condition to be treated. In particular, the absence of side effects and the impressive effectiveness of tetracosactide in the treatment of shock has been steadily confirmed in several clinical trials, so that it seems only right/imperative that tetracosactide be available in every ambulance and casualty ward, just like adrenaline and atropine. As previously discussed, the concordant results obtained in different animal models of ischemia and ischemia/reperfusion of single organs urgently ask for clinical studies. Should they confirm the data obtained in animals, it is foreseeable that melanocortins will

constitute a novel (and totally innovative) class of drugs for the treatment of such pathological conditions within the next 5–10 years.

The employment of melanocortin agonists or antagonists in the treatment of obesity or of anorexia and cachexia should usually be limited to a few months, and thus, the side effects should be acceptable; it should be the same for arthritic pathologies and neuropathic pain. In addition, occasional administrations, as in the case of sexual problems, are practically devoid of serious risks: only very few cases of exacerbation of preexisting hypertension have been reported. Similar is the case for the treatment of acute gout.

Highlights

The recent developments in the field of melanocortins point to:

- A definitively established therapeutic efficacy in multiple sclerosis, West's syndrome, gout attack, and nephrotic syndromes.
- A by now no more justified delay of the use (as intravenous bolus injection) of tetracosactide as first-aid, life-saving treatment 'in the field' in case of massively bleeding wounds and polytraumatisms, respiratory arrest, cardiac arrest, and any form of shock.
- The need for clinical trials that verify in humans the remarkable results obtained in several animal models of organ-limited ischemia or ischemia/reperfusion (especially stroke and myocardial infarction).
- Further efforts in the research for selective, well-tolerated, and efficacious MC4R agonists and antagonists for the treatment of obesity and cachexia (in addition to non-pharmacological treatments, when advisable).
- Further experimental verification in different animal models of the possible effect of melanocortins in neurodegenerative diseases and, in case, verification also in clinical setting.

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