ELSEVIER

European Journal of Pharmacology 405 (2000) 25–32

ejp

www.elsevier.nl/locate/ejphar

Role of melanocortins in the central control of feeding

Anna Valeria Vergoni *, Alfio Bertolini

Section of Pharmacology, Department of Biomedical Sciences, University of Modena and Reggio Emilia, via G. Campi 287, 41100 Modena, Italy

Accepted 28 June 2000

Abstract

The injection of a melanocortin peptide or of melanocortin peptide analogues into the cerebrospinal fluid or into the ventromedial hypothalamus in nanomolar or subnanomolar doses induces a long-lasting inhibition of food intake. The effect keeps significant for up to 9 h and has been observed in all animal species so far tested, the most susceptible being the rabbit. The anorectic effect of these peptides is a primary one, not secondary to the shift towards other components of the complex melanocortin-induced behavioral syndrome, in particular grooming. The site of action is in the brain, and the effect is not adrenal-mediated because it is fully exhibited also by adrenalectomized animals. It is a very strong effect, because the degree of feeding inhibition is not reduced in conditions of hunger, either induced by 24 h starvation, or by insulin-induced hypoglycemia, or by stimulation of γ-aminobutyric acid (GABA), noradrenergic or opioid systems. The microstructural analysis of feeding behavior suggests that melanocortins act as satiety-inducing agents, because they do not significantly modify the latencies to start eating, but shorten the latencies to stop eating. The mechanism of action involves the activation of melanocortin MC_4 receptors, because selective melanocortin MC_4 receptor antagonists inhibit the anorectic effect of melanocortins, while inducing per se a strong stimulation of food intake and a significant increase in body weight. Melanocortins seem to play an important role in stress-induced anorexia, because such condition, in rats, is significantly attenuated by the blockage of melanocortin MC_4 receptors; such a role is not secondary to an increased release of corticotropin-releasing factor (CRF), because, on the other hand, the CRF-induced anorexia is not affected at all by the blockage of melanocortin MC_4 receptors. The physiological meaning of the feeding inhibitory effect of melanocortins, and, by consequence, the physiological role of melanocortins in the complex machinery responsible for body weight homeostasis, is testified by the hyperphagia/obesity syndromes caused by mutations in the pro-opiomelanocortin (POMC) gene, or in the melanocortin MC_4 receptor gene, or in the agouti locus. Finally, recent evidences suggest that melanocortins could be involved in mediating the effects of leptin, and in controlling the expression of neuropeptide Y (NPY).

Keywords: Melanocortin; Feeding; Central nervous system; Behavior; Body weight homeostasis; Anorexia; Obesity

1. Introduction

The discovery that the injection of adrenocorticotrophic hormone (ACTH) or α-melanocyte stimulating hormone (α-MSH) into the cerebrospinal fluid or into defined brain areas (especially areas lining the third ventricle, and in particular the anteroventral quadrant of the third ventricle in the region of the organum vasculosum of the lamina terminalis), produces in mammals (monkeys, dogs, rats, rabbits, cats) a quite complex behavioral syndrome, was made by Ferrari et al. (1955). They hypothesized that ACTH and α-MSH might play a physiological role in central nervous system (CNS) functioning (Ferrari, 1958; Ferrari et al., 1963) long before the demonstration that indeed ACTH and α-MSH are synthesized not only by endocrine cells in the pituitary gland, but also by neurons in defined areas of the brain, and much longer before the discovery that receptors for melanocortin peptides are expressed in different regions of the CNS.

According to the description made by Ferrari and coworkers, the melanocortin-induced behavioral syndrome consisted of excessive grooming, and of repeated episodes of stretching and yawning. Oddly enough, the other main components of the syndrome (repeated episodes of penile erection, whole-body shakes, hyperalgesia, inhibition of feeding) have been noticed only several years later (Bertolini et al., 1969, 1975, 1979; Gispen and Isaacson,

* Corresponding author. Tel.: +39-59-2055372; fax: +39-59-2055376.
E-mail address: vergoni@unimo.it (A.V. Vergoni).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00538-0

1981; Vergoni et al., 1986, 1990). And only recently, taking advantage of the availability of superpotent melanocortin analogues, the long-questioned ability of these peptides to induce repeated episodes of penile erection (Bertolini et al., 1969, 1975) has been confirmed also in humans following subcutaneous administration of 0.015–0.025 mg/kg of melanotan-II (lactam cyclic [Nle^4, D-Phe^7] α-MSH (4–10) (Dorr et al., 1996; Wessells et al., 1998).

About twenty years ago, on the basis of anatomical, neurochemical and functional evidences, we put forward the hypothesis that many bodily functions are under the balanced control of an opioid–antiopioid system, the functionally far most important antiopioids being melanocortins (Bertolini et al., 1979, 1980, 1984, 1986a, 1988, 1989b, 1992; Bertolini and Ferrari, 1981; Bertolini, 1995). Such hypothesis has been shared by others (Hendrie, 1985; Alvaro et al., 1997; Fratta et al., 1981; Gessa et al., 1983). The experimental verification led to the discovery of several unforeseen activities of melanocortins (Bertolini et al., 1979, 1986b; Castelli et al., 1985): among them, the feeding-inhibitory effect (Vergoni et al., 1986; Poggioli et al., 1986).

2. The feeding-inhibitory effect of melanocortins

The injection of ACTH-(1–24) into a brain lateral ventricle after a 24 h starvation period, or into the ventromedial hypothalamus during the nocturnal feeding phase, markedly inhibits food intake in rats. In starved rats, the dose of 4 μg/animal is maximally effective and reduces food intake by 70–80% during the first hour after treatment. The same dose, injected into the ventromedial hypothalamus, also inhibits food intake in freely feeding rats during the nocturnal phase (almost 60% reduction during the 90 min of observation) (Vergoni et al., 1986). The inhibitory effect of ACTH-(1–24) on feeding has been confirmed also in mice and rabbits (Bertolini et al., 1988). In these last experiments, animals were observed for 4 h after treatment, and the feeding-inhibitory effect of ACTH-(1–24) lasted, and kept quite stable, for the whole observation period. The maximum effect was obtained with a dose of 0.05 μg/animal in mice (about 60% reduction of food intake during the overall 4 h of observation) and of 10 μg/animal in rabbits (about 73% reduction during the overall observation period). This study showed that the duration of the anorectic effect far exceeds that of the other behavioral effects of ACTH. Indeed, in rats, grooming lasts for about 1 h after treatment, and stretching and yawning last for about 2 h (Bertolini et al., 1988). Thus, it seems unlikely that the possible inhibitory influence on feeding of the other behaviors (particularly grooming and stretching) may play a role in the overall reduction of food intake. This is further supported by the fact that rabbits are the most markedly affected by the anorectic activity of ACTH, while being the least susceptible to the grooming stimulant effect (Bertolini et al., 1988). In other studies, (Poggioli et al., 1986) food intake was measured in rats for up to 9 h after the intracerebroventricular (i.c.v.) injection of ACTH-(1–24) or α-MSH. Both peptides markedly inhibited spontaneous feeding during the whole period of observation. At the doses of 4 or 10 μg/rat, ACTH-(1–24) and α-MSH abolished the feeding-stimulatory effect of the kappa opiate receptor agonist pentazocine, intraperitoneally (i.p.) injected at the dose of 10 mg/kg.

The same antagonism was obtained by the i.c.v. injection of ACTH-(1–24) (4 μg/rat) into rats i.p. treated with two other kappa opiate agonists, bremazocine and tifluadom, at the doses of 1 and 5 mg/kg, respectively (Poggioli et al., 1986).

The site of action of melanocortins for their feeding-inhibitory effect is in the CNS. Indeed, the subcutaneous administration of ACTH-(1–24) is without effect on feeding behavior up to the dose of 200 μg/kg (Vergoni et al., 1990), which is, on the other hand, maximally effective in producing other effects of peripherally administered ACTH, e.g., corticotrophic activity (Atcheson and Tyler, 1975), antagonism of the cholestatic and constipating effects of morphine (Poggioli et al., 1988), and reversal of a severe condition of hemorrhagic shock (Bertolini et al., 1986a,b, 1989a). This indicates that the anorectic effect of ACTH is not linked to an interaction with the peripheral feeding-regulatory system. Moreover, the feeding-inhibitory activity of ACTH-(1–24) is not affected by adrenalectomy, indicating that it is not linked to the release of adrenal steroids (Vergoni et al., 1990).

The anorectic effect of ACTH is extremely strong as it is observed not only after 24 h of food deprivation, but also in the presence of other stimuli known to cause vigorous feeding, such as insulin-induced hypoglycemia, and stimulation of the γ-aminobutyric acid (GABA) (muscimol, 250 ng/rat i.c.v.) or noradrenergic (norepinephrine, 20 μg/rat i.c.v.) systems (Vergoni et al., 1990).

The microstructural analysis of feeding behavior (according to Kirkham and Blundell, 1984) indicates that melanocortins are more likely to act as satiety-inducing, rather than as hunger-reducing agents. Indeed, the i.c.v. injection of ACTH-(1–24) into rats fasted for 24 h invariably shortens the latencies to stop eating without significantly influencing the latencies to start eating (Vergoni et al., 1990).

Additional evidence of the feeding-inhibitory effect of melanocortins was provided several years later (Fan et al., 1997). These authors confirmed our previous data by i.c.v. injecting into mice the cyclic lactam melanocortin agonist melanotan-II. In mice deprived of food for 16 h before treatment, melanotan-II dose-dependently inhibited food intake for up to 4 h after administration, and normal feeding rates resumed about 8 h after treatment. The i.c.v. administration of melanotan-II also reduced food intake in

three other models of hyperphagia: *ob/ob* mice, A^y (agouti yellow) mice, and neuropeptide Y (NPY) treated mice. Melanotan-II also significantly inhibited normal nocturnal food intake.

Following i.p. administration, melanotan-II significantly reduced food intake only for doses 30–40 times higher than those effective by the i.c.v. route of administration, thus confirming that melanocortins inhibit feeding by acting in the brain.

3. Mechanism of action

With the discovery and cloning of the melanocortin receptors (Chhajlani and Wikberg, 1992; Chhajlani et al., 1993; Mountjoy et al., 1992; Gantz et al., 1993, 1994; Adan et al., 1994; for reviews see: Wikberg, 1999) and the ensuing availability of selective antagonists, the molecular mechanisms underlying the various effects of melanocortins have started to become elucidated.

The melanocortin receptors belong to the G-protein coupled receptor family, and all of them couple in a stimulatory fashion to adenylyl cyclase. The melanocortin receptors show distinct distributions in the body. The melanocortin MC_1 receptor was first recognized as the peripheral MSH receptor which is present in melanocytes, where it regulates the pigmentation of the skin. But melanocortin MC_1 receptor is also present in other tissues besides the skin: periaqueductal grey matter, pituitary, Leydig cells of the testis, corpus luteum, trophoblastic cells of the placenta, macrophages and monocytes, neutrophils, endothelial cells, glioma cells and astrocytes.

The melanocortin MC_2 receptor is the ACTH receptor. It is highly expressed in the cortex of the adrenal gland, where it mediates the hormonal corticotrophic effect of ACTH. The densest expression occurs in the zona reticularis and fasciculata, with the expression in the zona reticularis being less pronounced. A few scattered cells of the adrenal medulla also express the melanocortin MC_2 receptor. Besides the adrenal gland, the melanocortin MC_2 receptor is expressed in the white adipose tissue of mice (but not of humans), and in the skin (ACTH and ACTH fragments are produced in the epidermis, and ACTH induces DNA synthesis and cell proliferation of keratinocytes).

The melanocortin MC_3 receptor is primarily found in the CNS. However, it is also expressed in the placenta, gut and heart. In the CNS, the highest densities are found in regions of the hypothalamus and limbic system. Very high densities of the melanocortin MC_3 receptors are present in particular in the ventromedial nucleus of the hypothalamus and the nucleus accumbens. The melanocortin MC_3 receptor is also found in the septum, hippocampus, thalamus, and midbrain including the ventral tegmental area. [The dominance of melanocortin MC_3 receptors in the nucleus accumbens is of interest in relation to a repeatedly reported connection of the melanocortin system in opiate addiction (Jacquet, 1978; Bertolini and Ferrari, 1981; Bertolini et al., 1984; Gessa et al., 1983; Hendrie, 1985; Mucha and van Ree, 1989; Alvaro et al., 1997), other forms of drug addiction, as well as possible roles for the melanocortin MC_3 receptors in psychiatric diseases (Wikberg, 1999)].

The melanocortin MC_4 receptor is by far the most abundant and most widely distributed melanocortin receptor subtype in the brain. It is expressed in multiple sites in virtually every CNS region including the cortex, thalamus, hypothalamus, brainstem, and spinal cord; the highest concentrations being found in the septum, nucleus accumbens, neostriatum, periaqueductal grey and hypothalamus. It is not detectable in the periphery, with the exception of chickens, where it is expressed in many peripheral tissues. The melanocortin MC_4 receptor expression is predominant during the whole foetal period: this is of interest, in view of the potent trophic effect of α-MSH on the foetal brain (for review see: O'Donohue and Dorsa, 1982).

The melanocortin MC_5 receptor expression is ubiquitous, its mRNA being detected in many peripheral tissues, including several exocrine glands and endocrine organs and white adipose cells. Melanocortin MC_5 receptor mRNA has also been shown to be expressed at extremely low levels in several brain regions.

Recently accumulated data suggest that the inhibitory effect of melanocortins on feeding is mediated mainly (and probably exclusively) by central melanocortin MC_4 receptors. The targeted disruption of the melanocortin MC_4 receptor results in obesity in mice (Huszar et al., 1997). These animals develop a maturity-onset obesity, with about 50% increased food consumption and body weight at 15 weeks of age, as well as an about 10% increase in body length. This obesity syndrome is associated with hyperphagia, hyperinsulinemia, and hyperglicemia. The development and the availability of selective melanocortin receptor-antagonists has further confirmed the role of melanocortin MC_4 receptors in feeding behavior. One of these compounds, HS014 (cyclic [$AcCys^{11}$, D-Nal^{14}, Cys^{18}, Asp-NH_2^{22}]-β-MSH-(11–22), is a cyclic α-MSH analogue with 34-, 17- and 220-fold selectivity for the melanocortin MC_4 receptor over the melanocortin MC_1, MC_3 and MC_5 receptors, respectively (Schiöth et al., 1998); moreover, it is a low-affinity partial agonist of the melanocortin MC_1 receptor and the melanocortin MC_5 receptor. The acute i.c.v. administration of HS014 in the dose range of 0.33–3.33 nmol/animal during the daytime, when food intake is generally low, dose-dependently increased food intake in non-starved rats: at 4 h after the administration of 1 nmol, cumulative food intake was increased by 100% (Kask et al., 1998c). The orexygenic effect of HS014 has been observed also after 18 h of starvation. While α-MSH reduced the time spent feeding by approximately 50%, HS014 increased it by approximately 60%. When α-MSH and HS014 were given together, the feeding time was not different from that of control rats (Vergoni et al., 1998).

The impressive stimulatory effect of HS014 on food intake has also been observed following chronic administration: either i.c.v. injected twice daily (1 nmol × 2) for 6 days, or administered by continuous i.c.v. infusion with osmotic minipumps (0.16 nmol/h) for 2 weeks. HS014 induced a considerable increase in food intake and body weight after either treatment without any sign of tachyphylaxis (Kask et al., 1999). After 2 weeks of continuous i.c.v. infusion, the HS014-treated rats showed a 20% increase in body weight in comparison with rats i.c.v. infused with saline.

Another study (Vergoni et al., 2000) confirmed the above-quoted data and showed that sexual behavior of males was not affected. When the infusion of HS014 was terminated and pumps disconnected, the food intake gradually decreased, and, 4 days after the end of the infusion, it was not significantly different from that of saline-infused rats. The rats that had been treated with HS014 continued to lower their food intake, and starting from the 10th day after the end of the infusion, the daily food intake was significantly lower than in controls. In parallel, rats that had been chronically i.c.v. infused with HS014 gradually lost weight after treatment termination and minipump disconnection, tending to the weight of controls. These results indicate that overeating and consequent increase in body weight caused by melanocortin MC_4 receptor blockage is reversible when the blockage is removed.

These data moreover suggest that the melanocortin control of food intake is very robust, and that changes induced by such treatment overcome negative feedback signals. Other selective melanocortin MC_4 receptor antagonists, HS024 (cyclic [$AcCys^3$, Nle^4, Arg^5, D-Nal^7, Cys-NH_2^{11}] α-MSH-(3–11) and HS028 (cyclic [$AcCys^{11}$, dichloro-D-phenylalanine^{14}, Cys^{18}, Asp-NH_2^{22}]-β-MSH-(11-22), gave similar results. HS024 caused a dramatic (i.e., up to 160%), dose-dependent increase in food intake in free feeding rats, after i.c.v. administration (Kask et al., 1998a). HS028, which shows higher affinity and selectivity for the melanocortin MC_4 receptors compared to HS014 (Skuladottir et al., 1999), chronically i.c.v. infused by subcutaneously implanted osmotic minipumps, significantly increased both food intake and body weight in a dose-dependent manner, without signs of tachyphylaxis during the 7 days treatment (Skuladottir et al., 1999).

4. Role of melanocortins in eating disorders

The agouti protein, normally only expressed in the skin, is a high-affinity antagonist of the MSH receptor (melanocortin MC_1 receptor), thus explaining its inhibitory effect on eumelanin pigment synthesis. It is also a competitive antagonist of the melanocortin MC_4 receptor (Lu et al., 1994).

Dominant mutations of the agouti locus that result in widespread ectopic expression of the agouti protein cause the pleiotropic agouti obesity syndrome (obese yellow mouse; mouse lethal yellow mutation) (Duhl et al., 1994; Michaud et al., 1994), characterized by hyperphagia, hyperinsulinaemia, late-onset obesity. I.c.v. administration of a melanocortin MC_3/MC_4 receptor agonist peptide inhibits food intake in those animals, suggesting that their obesity state results from the cumulative effect of chronic antagonism of melanocortin MC_4 receptor signalling in the brain by agouti protein (Fan et al., 1997).

A structurally related protein known as the agouti-related protein (AGRP) is normally expressed in the brain and acts as an antagonist of the melanocortin MC_3 and MC_4 receptors. Over-expression of AGRP in transgenic mice also leads to hyperphagia and obesity (Ollmann et al., 1997). It is likely that AGRP functions as an endogenous melanocortin receptor antagonist that regulates central melanocortin neurotransmission.

A quite superimposable condition of hyperphagia and obesity is obtained in knockout mice lacking the melanocortin MC_4 receptor (Huszar et al., 1997). And quite similar syndromes have been recently described and characterized in human beings that have mutations in the pro-opiomelanocortin (POMC) gene, leading to deficiency in ACTH and MSH (severe early onset obesity, hyperphagia, adrenal insufficiency and red hair pigmentation) (Krude et al., 1998) or that have mutations in the melanocortin MC_4 receptor gene (dominant form of obesity) (Vaisse et al., 1998; Yeo et al., 1998).

The influence of stress on feeding behavior, both in animals and humans, is well known (Morley et al., 1986; Slochower, 1976; Troop and Treasure, 1997). The resulting effect can vary considerably depending on the nature of the stress. Both the type and the duration of the stress appear to play a role in determining the effect on food intake. Thus, mild tail-pinching produces overeating in the rat, while severe stress, such as immobilization or exposure to a novel environment leads to anorexia (Morley et al., 1983). It has also been postulated that psycho-social stress may be an important precipitating factor in the etiology of anorexia nervosa and that the activation of the opioid and hypothalamic–pituitary–adrenal systems indicates that anorexia nervosa may be a stress syndrome (Donohoe, 1984; Gillmann and Lichtigfeld, 1983; Gold et al., 1986; Hotta et al., 1986). Significant body weight loss has been reported to occur in animals after exposure to highly stressful conditions (Pare, 1965). A stressful situation which induces severe anorexia and a decrease in body weight in rats is repeated immobilization (Grignaschi et al., 1993; Shimizu et al., 1989). Restraint stress has also been proposed as a behavioral model of anorexia nervosa (Haslam et al., 1987) on the basis of the reported evidence that psychological stress may play an important role in this disorder (Donohoe, 1984).

Experiments aimed at investigating whether immobilization stress-induced anorexia may be affected by acute or chronic blockage of melanocortin MC_4 receptors have

been performed. Rats were stressed by strapping their paws to restraining grids with plastic clamps. Immobilization lasted 30 min on days 1 and 2, and 15 min on days 3 and 4. On days 1–4, the animals were i.c.v. injected with the melanocortin MC_4 selective antagonist HS014, at the dose level of 10 μg/rat, 5 min before starting the immobilization period. The amount of food eaten was measured, 1, 2 and 3 h after the immobilization, and again 22 h later. The effect of stress in reducing food intake was significant at all the above times: stressed rats always ate less than 50% of the food compared with the control non-stressed rats. The daily average food intake was 26 g/rat for the control group and 10–12 g/rat for the stressed group. The rats which were stressed and treated with HS014 had, in all cases, a higher food intake than the stressed untreated rats. This difference was, however, only significant at the 22 h recording point, and not significant during the first 3 h after immobilization. The 22 h data showed that the stressed HS014-treated rats ate significantly less than the non-stressed control rats, but significantly more than the stressed untreated rats on days 1 and 2. On days 3, 4 and 5 (first day without stress and treatment), the stressed HS014-treated animals had a significantly higher food intake than the stressed untreated rats, and a not significantly lower food intake than the non-stressed controls. HS014 also prevented in part the loss of body weight associated to the immobilization stress-induced anorexia. Body weight was significantly lower in the stressed untreated group than in the control group on days 2, 3, 4 and 5. The stressed group treated with HS014 had a significantly lower body weight than the non-stressed control group, but a higher body weight than the stressed untreated group on days 3, 4 and 5. On day 5, the stressed untreated group had a 13% lower body weight than the non-stressed control group, while the stressed HS014-treated group had a 5.6% lower body weight than the non-stressed control group (Vergoni et al., 1999a).

It is thus evident that melanocortin MC_4 receptor blockage can reduce stress-induced anorexia and that repeated injections of HS014 have a sustained effect on food intake without any sign of tachyphylaxis. The effect is, however, only partial, indicating that stress-induced anorexia is not solely mediated through melanocortin MC_4 receptors. This is fairly conceivable, considering the complex nervous–endocrine–autonomic response to stressful situations. Indeed, it has been shown that restraint stress-induced feeding inhibition is also partially reversed by pretreatment with corticotropin releasing factor (CRF) antagonists, producing evidence in support of the idea that CRF, too, is involved in the inhibitory mechanism of food intake in restraint stress (Krahn et al., 1986; Shibasaki et al., 1988). It could be surmised that such effect of CRF may be indirectly mediated by the release of melanocortin peptides from POMC neurons. However, this is not the case. In fact, we have recently shown that the significant reduction of food intake, feeding time and feeding episodes produced by the i.c.v. injection of CRF (3 μg/rat) is not at all influenced by the melanocortin MC_4 receptor antagonist, HS014 (Vergoni et al., 1999b).

5. The place of melanocortins in the feeding regulatory system

Leptin — the protein hormone produced mainly by white adipose tissue that conveys to the brain information about the size of energy stores and activates hypothalamic centres that regulate energy intake and expenditure (Flier, 1997; Mantzoros and Moschos, 1998) — increases the expression of proopiomelanocortin mRNA in the rostral arcuate nucleus (Schwartz et al., 1997). The leptin signalling on the arcuate nucleus appears to be directed to the paraventricular nucleus of the hypothalamus as the administration of leptin leads to increased c-*fos* expression in that area. Such effect of leptin is blocked by the melanocortin MC_3/MC_4 receptor antagonist SHU9119 (cyclic [Nle^4, Asp^5, D-Nal^7, Lys^{10}]α-MSH-(4–10) (Seeley et al., 1997). And the selective melanocortin MC_4 receptor antagonist HS014 significantly attenuates the feeding inhibition and the loss of body weight induced by leptin in rats (Kask et al., 1998d). These data thus indicate that POMC neurons originating in the arcuate nucleus contacts synaptically at melanocortin MC_4 receptor containing neurons (in the paraventricular nucleus or other hypothalamic nuclei) that participate in the control of feeding motivation. This POMC-related melanocortin MC_4 receptor signalling thus appears clearly to occur downstream to the leptin signalling. This is also supported by the observation that the melanotan-II-induced anorectic effect has been seen also in leptin deficient C57BL/6JLep^{ob} mice (Fan et al., 1997). Thus, it seems that leptinergic and melanocortinergic signals act synergically, at least at the central level, and that leptin acts in the brain through the stimulation and/or potentiation of melanocortinergic transmission upon hypothalamic centres involved in feeding control. Moreover, it has been shown in normal rats that the i.c.v. administration of melanotan-II (a MC_3/MC_4 agonist) stimulates in a dose-dependent manner the sympathetic outflow to brown adipose tissue (Haynes et al., 1999). SHU9119 (a potent MC_3/MC_4 antagonist), on the other hand, prevents also the increase in mRNA UCP (uncoupling proteins) expression induced by leptin in brown adipose tissue.

These evidences suggest that melanocortins could be involved not only in the CNS, but also in the periphery in mediating the effects of leptin (Satoh et al., 1998). Moreover, these same evidences may suggest that the intense change in body weight observed during the chronic administration either of melanocortins or of their antagonists can be linked also to a direct peripheral effect on energy expenditure in the brown adipose tissue by means of regulating the mRNA UCP expression.

The melanocortinergic system also interacts with NPY. Indeed, while normal mice lack detectable mRNA expression of the NPY gene in neurons of the medial hypothalamic nucleus, an intense expression occurs in the lethal yellow agouti mice as well as in melanocortin MC_4 receptor knockouts (Kesterson et al., 1997). Moreover, it has been shown that the NPY1 receptor blocker 1229U91 attenuates the feeding-stimulatory effect of the melanocortin MC_4 receptor antagonist HS014 (Kask et al., 1998b). The combined data would suggest that melanocortins inhibit the expression of NPY in the medial hypothalamic nucleus, an effect mediated by melanocortin MC_4 receptors.

6. Concluding remarks

Our findings concerning the feeding-inhibitory effect of melanocortins (Vergoni et al., 1986, 1990; Poggioli et al., 1986) have been entirely confirmed by the subsequent studies. The discovery of the melanocortin receptors, the availability of transgenic and knockout animals, the synthesis of melanocortin antagonists and superagonists highly selective for the different receptors subtypes, have led to a better understanding of the role of melanocortins in feeding control and in body weight and energy expenditure homeostasis.

The exponential growth of research in the field of feeding disorders during the last decade has led to the discovery of other regulators of food intake and energy homeostasis (a wide array that includes leptin, ciliary neurotrophic factor, orexins, hypocretin, melanin concentrating hormone, oxytocin, interleukins 1 and 6, tumor necrosis factor-α (TNF-α), etc.): because of the obvious importance of feeding for survival, it is not surprising that feeding behavior is controlled by redundant mechanisms. The emerging relationships with other main regulators of food intake (opioids, leptin, NPY, oxytocin) have stressed the crucial importance of melanocortins in feeding and weight homeostasis.

Obesity is an increasingly worrying health problem for an increasingly large part of mankind (on the other hand, underfeeding is an increasingly tremendous problem for another increasingly large part of mankind). Obesity is associated with the development of some of the most prevalent diseases of modern society. In Europe, the mean prevalence of overweight + obese men (35–64 years of age) is 64.2 ± 6.8 (mean ± S.D.). In US subgroups of populations (black, Hispanic and mid-American women) the prevalence of clinical obesity exceeds 50% (Seidell and Flegal, 1997). Anorectic conditions leading to cachexia are as well a most serious disease.

The recent developments in feeding research should not only elucidate the pathophysiology of energy intake and homeostasis, but will also hopefully results in effective, mechanism-based treatments for obesity and anorexia. The last experimental data on melanocortin agonists and antagonists are encouraging steps in this direction.

Acknowledgements

The experimental portion of this research was supported by Italian M.U.R.S.T. We thank Doctor Gustavo Savino for his helpful assistance in writing the manuscript.

References

Adan, R.A., Oosterom, J., Ludvigsdottir, G., Brakkee, J.H., Burbach, J.P.H., Gispen, W.H., 1994. Identification of antagonists for melanocortin MC_3, MC_4 and MC_5 receptors. Eur. J. Pharmacol. 269, 331–337.

Alvaro, J.D., Tartro, J.B., Duman, R.S., 1997. Melanocortins and opiate addiction. Life Sci. 61, 1–9.

Atcheson, J.B., Tyler, F.H., 1975. Circadian rhythm: man and animals. In: Blasatko, H., Sayers, G., Smith, A.D. (Eds.), Handbook of Physiology: Adrenal Glands vol. 6 American Physiological Society, Washington, pp. 127–145, Section 7.

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

Bertolini, A., Ferrari, W., 1981. Evidence and implication of a melanocortins–endorphins homeostatic system. In: Endroczi, E. (Ed.), Neuropeptides and Psychosomatic Processes. Academiai Kiadò, Budapest, pp. 245–261.

Bertolini, A., Vergoni, W., Gessa, G.L., Ferrari, W., 1969. Induction of sexual excitement by the action of adrenocorticotrophic hormone in brain. Nature 221, 667–669.

Bertolini, A., Gessa, G.L., Ferrari, W., 1975. Penile erection and ejaculation: a central effect of ACTH-like peptides in mammals. In: Sandler, M., Gessa, G.L. (Eds.), Sexual Behavior: Pharmacology and Biochemistry. Raven Press, New York, pp. 247–257.

Bertolini, A., Poggioli, R., Ferrari, W., 1979. ACTH-induced hyperalgesia in rats. Experientia 35, 1216–1217.

Bertolini, A., Poggioli, R., Ferrari, W., 1980. Possible physiological role of ACTH-peptides in nociception. Adv. Biochem. Psychopharmacol. 22, 109–117.

Bertolini, A., Fratta, W., Melis, M., Gessa, G.L., 1984. Possible role of ACTH–MSH peptides in morphine tolerance and withdrawal in rats. In: Biggio, G., Spano, P.F., Toffano, G., Gessa, G.L. (Eds.), Neuromodulation and Brain Function. Pergamon, Oxford, pp. 225–230.

Bertolini, A., Guarini, S., Rompianesi, E., Ferrari, W., 1986a. α-MSH and other ACTH fragments improve cadiovascular function and survival in experimental hemorrhagic shock. Eur. J. Pharmacol. 139, 19–26.

Bertolini, A., Poggioli, R., Vergoni, A.V., Castelli, M., Guarini, S., 1986b. Evidence that melanocortins are physiological antagonists of opioids. In: De Wied, D., Ferrari, W. (Eds.), Central Actions of ACTH and Related Peptides. Liviana Press, Padova, pp. 207–222.

Bertolini, A., Poggioli, R., Vergoni, A.V., 1988. Cross-species comparison of the ACTH-induced behavioral syndrome. Ann. N. Y. Acad. Sci. 525, 114–129.

Bertolini, A., Ferrari, W., Guarini, S., 1989a. The adrenocorticotropic hormone (ACTH)-induced reversal of hemorrhagic shock. Resuscitation 18, 253–267.

Bertolini, A., Poggioli, R., Guarini, S., Genedani, S., Vergoni, A.V., 1989b. Endogenous antagonists of opioid peptides. In: Genazzani, A.R., Negri, M. (Eds.), Opioid Peptides in Biological Fluids. Parthenon Publishing Group, Lancaster, pp. 33–43.

Bertolini, A., Poggioli, R., Arletti, R., Benelli, A., Marrama, D., Bazzani, C., Tagliavini, S., Bernardi, M., Rasori, E., Sandrini, M., Guarini, S., Genedani, S., Vergoni, A.V., 1992. Anatomia chimica e funzionale dei sistemi peptidergici. In: Gori, E., Muller, E.E. (Eds.), Basi Biologiche e Farmacologiche delle Tossicodipendenze. Pythagora Press, Milan, pp. 25–76.

Castelli, M., Gessa, G.L., Bertolini, A., 1985. ACTH-(1–24) antagonizes the contractile effect of morphine on the isolated rat colon. Eur. J. Pharmacol. 108, 213–214.

Chhajlani, V., Wikberg, J.E.S., 1992. Molecular cloning and expression of the human melanocyte stimulating hormone receptor cDNA. FEBS Lett. 309, 417–420.

Chhajlani, V., Muceniece, R., Wikberg, J.E.S., 1993. Molecular cloning of a novel human melanocortin receptor. Biochem. Biophys. Res. Commun. 195, 866–873.

Donohoe, T.P., 1984. Stress-induced anorexia: implications for anorexia nervosa. Life Sci. 34, 203–218.

Dorr, R.T., Lines, R., Levine, N., Brooks, C., Xiang, L., Hruby, V.J., Hadley, M.E., 1996. Evaluation of melanotan-II, a superpotent cyclic melanotropic peptide in a pilot phase-I clinical study. Life Sci. 58, 1777–1784.

Duhl, D.M.J., Vrieling, H., Miller, K.A., Wolff, G.L., Barsh, G.S., 1994. Neomorphic agouti mutations in obese yellow mice. Nat. Genet. 8, 59–65.

Fan, W., Boston, B.A., Kesterson, R.A., Hruby, V.J., Cone, R.D., 1997. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 385, 165–168.

Ferrari, W., Floris, E., Paulesu, F., 1955. Su di una particolare, imponente sintomatologia prodotta nel cane dall'ACTH iniettato nella cisterna magna. Boll. Soc. Ital. Biol. Sper. 31, 862–864.

Ferrari, W., 1958. Behavioral changes in animals after intracisternal injection with adrenocorticotrophic hormone and melanocyte-stimulating hormone. Nature 181, 925–926.

Ferrari, W., Gessa, G.L., Vargiu, L., 1963. Behavioral effects induced by intracisternally injected ACTH and MSH. Ann. N. Y. Acad. Sci. 104, 330–345.

Flier, J.S., 1997. Leptin expression and action: new experimental paradigms. Proc. Natl. Acad. Sci. U. S. A. 94, 4242–4245.

Fratta, W., Rossetti, Z.L., Poggioli, R., Gessa, G.L., 1981. Reciprocal antagonism between ACTH 1–24 and beta-endorphin in rats. Neurosci. Lett. 24, 71–74.

Gantz, I., Konda, Y., Tashiro, T., Shimoto, Y., Miwa, H., Munzert, G., Watson, S.J., DelValle, J., Yamada, T., 1993. Molecular cloning of a novel melanocortin receptor. J. Biol. Chem. 268, 8246–8250.

Gantz, I., Shimoto, Y., Konda, Y., Miwa, H., Dickinson, C.J., Yamada, T., 1994. Molecular cloning, expression and characterization of a fifth melanocortin receptor. Biochem. Biophys. Res. Commun. 200, 1214–1220.

Gessa, G.L., Fratta, W., Melis, M., Bertolini, A., Ferrari, W., 1983. Hypothalamic ACTH and MSH levels increase in morphine tolerance and decrease after morphine withdrawal. Eur. J. Pharmacol. 95, 143–144.

Gillmann, M.A., Lichtigfeld, E., 1983. The opioid system and anorexia nervosa. Am. J. Psychiatry 140, 371–372.

Gispen, W.H., Isaacson, R.L., 1981. ACTH-induced excessive grooming in the rat. Pharmacol. Ther. 12, 209–246.

Gold, P.W., Gwirtsman, H., Avgerinos, P.C., Nieman, L.K., Gallucci, W.T., Kaye, W.H., Jimerson, D., Ebert, M., Rittmaster, R., Loriaux, D.L., Chrousos, G.P., 1986. Abnormal hypothalamic–pituitary–adrenal function in anorexia nervosa: pathophysiological mechanisms in underweight and weight-corrected patients. N. Engl. J. Med. 314, 1335–1342.

Grignaschi, G., Mantelli, B., Samanin, R., 1993. The hypophagic effect of restraint stress in rats can be mediated by 5-HT2 receptors in the paraventricular nucleus of the hypothalamus. Neurosci. Lett. 152, 103–106.

Haslam, C., Stevens, R., Donohoe, T.P., 1987. The influence of cyproheptadine on immobilization and oestradiol benzoate induced anorexia in ovariectomized rats. Psychopharmacology 93, 201–206.

Haynes, W.G., Morgan, D.A., Djalali, A., Sivitz, W.I., Mark, A.L., 1999. Interactions between the melanocortin system and leptin in control of sympathetic nerve traffic. Hypertension 33, 542–547.

Hendrie, C.A., 1985. Opiate dependence and withdrawal — a new synthesis? Pharmacol. Biochem. Behav. 23, 863–868.

Hotta, M., Shibasaki, T., Masuda, A., Imaki, T., Demura, H., Ling, N., Shizume, K., 1986. The response of plasma adrenocorticotropin and cortisol to corticotropin-releasing hormone (CRH) and cerebrospinal fluid immunoreactive CRH in anorexia nervosa patients. J. Clin. Endocrinol. Metab. 62, 319–324.

Huszar, D., Lynch, C.A., Faurchild-Huntress, V., Dumore, J.H., Fang, Q., Berkemeyer, L.R., Gu, W., Kesterson, R.A., Boston, B.A., Cone, R.D., Smith, F.J., Campfield, L.A., Burn, P., Lee, F., 1997. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88, 131–141.

Jacquet, Y.F., 1978. Opiate effects after adrenocorticotropin or beta-endorphin injection in the periaqueductal gray matter of rats. Science 201, 1032–1034.

Kask, A., Mutulis, F., Muceniece, R., Pahkla, R., Mutule, I., Wikberg, J.E., Rago, L., Schioth, H.B., 1998a. Discovery of a novel superpotent and selective melanocortin-4 receptor antagonist (HS024): evaluation in vitro and in vivo. Endocrinology 139, 5006–5014.

Kask, A., Rago, L., Korrovits, P., Wikberg, J.E., Schioth, H.B., 1998b. Evidence that orexigenic effects of melanocortin 4 receptor antagonist HS014 are mediated by neuropeptide Y. Biochem. Biophys. Res. Commun. 248, 245–249.

Kask, A., Rago, L., Mutulis, F., Pahkla, R., Wikberg, J.E., Schioth, H.B., 1998c. Selective antagonist for the melanocortin 4 receptor (HS014) increases food intake in free-feeding rats. Biochem. Biophys. Res. Commun. 245, 90–93.

Kask, A., Rago, L., Wikberg, J.E., Schioth, H.B., 1998d. Evidence for the involvement of the melanocortin MC_4 receptor in the effects of leptin on food intake and body weight. Eur. J. Pharmacol. 360, 15–19.

Kask, A., Pahkla, R., Irs, A., Rago, L., Wikberg, J.E., Schioth, H.B., 1999. Long-term administration of MC_4 receptor antagonist HS014 causes hyperphagia and obesity in rats. NeuroReport 10, 707–711.

Kesterson, R.A., Huszar, D., Lynch, C.A., Simerly, R.B., Cone, R.D., 1997. Induction of neuropeptide Y gene expression in the dorsal medial hypothalamic nucleus in two models of the agouti obesity syndrome. Mol. Endocrinol. 11, 630–637.

Kirkham, T.C., Blundell, J.E., 1984. Dual action of naloxone on feeding revealed by behavioral analyses: separate effects on initiation and termination of eating. Appetite 5, 45–52.

Krahn, D.D., Gosnell, B.A., Grace, M., Levine, A.S., 1986. CRF antagonist partially reverses CRF- and stress-induced effects on feeding. Brain Res. Bull. 17, 285–289.

Krude, H., Biebermann, H., Luck, W., Horn, R., Brabant, G., Gruters, A., 1998. Severe early onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. Nat. Genet. 19, 155–157.

Lu, D., Willard, D., Patel, I.R., Kadwell, S., Overton, L., Kost, T., Luther, M., Chen, W., Woychik, R.P., Wilkinson, W.O., Cone, R.D., 1994. Agouti protein is an antagonist of the melanocyte-stimulating hormone receptor. Nature 371, 799–802.

Mantzoros, C.S., Moschos, S.J., 1998. Leptin: in search of role(s) in human physiology and pathophysiology. Clin. Endocrinol. (Oxford) 49, 551–567.

Michaud, E.J., Bultman, S.J., Klebig, M.L., Van Vugt, M.J., Stubbs, L.J., Russell, L.B., Woychik, R.P., 1994. A molecular model for the genetic and phenotypic characteristics of the mouse lethal yellow (*Ay*) mutation. Proc. Natl. Acad. Sci. U. S. A. 91, 2562–2566.

Morley, J.E., Levine, A.S., Rowland, N.E., 1983. Stress induced eating. Life Sci. 32, 2169–2182.

Morley, J.E., Levine, A.S., Willenbring, M.L., 1986. Stress-induced feeding disorders. In: Carruba, M.O., Blundell, J.E. (Eds.), Pharma-

cology of Eating Disorders: Theoretical and Clinical Developments. Raven Press, New York, pp. 71–83.

Mountjoy, K.G., Robbins, L.S., Mortrud, M.T., Cone, R.D., 1992. The cloning of a family of genes that encode the melanocortin receptors. Science 257, 1248–1251.

Mucha, R.F., Van Ree, J.M., 1989. Infusion of gamma 2-MSH produce a conditioned taste aversion in morphine-dependent rats. Psychopharmacology 99, 140–142.

O'Donohue, T.L., Dorsa, D.M., 1982. The opiomelanotropinergic neuronal and endocrine systems. Peptides 3, 353–395.

Ollmann, M.M., Wilson, B.D., Yang, Y.-K., Kerns, J.A., Chen, Y., Gantz, I., Barsh, G.S., 1997. Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. Science 278, 135–138.

Pare, W.P., 1965. Stress and consummatory behavior in the albino rat. Psychol. Rev. 16, 399–405.

Poggioli, R., Vergoni, A.V., Bertolini, A., 1986. ACTH-(1–24) and α-MSH antagonize feeding behavior stimulated by kappa opiate agonists. Peptides 7, 843–848.

Poggioli, R., Arletti, R., Vergoni, A.V., Castelli, M., Bertolini, A., 1988. ACTH-(1–24) antagonizes the cholestatic and constipating effects of morphine. Arch. Int. Pharmacodyn. Ther. 293, 265–272.

Satoh, N., Ogawa, Y., Katsuura, G., Numata, Y., Masuzaki, H., Yoshimasa, Y., Nakao, K., 1998. Satiety effect and symapthetic activation of leptin are mediated by hypothalamic melanocortin system. Neurosci. Lett. 249, 107–110.

Schiöth, H.B., Mutulis, F., Muceniece, R., Prusis, P., Wikberg, J.E., 1998. Discovery of novel melanocortin 4 receptor selective MSH analogues. Br. J. Pharmacol. 124, 75–82.

Schwartz, M.W., Seeley, R.J., Woods, S.C., Weigle, D.S., Campfield, L.A., Burn, P., Baskin, D.G., 1997. Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes 46, 2119–2123.

Seeley, R.J., Yagaloff, K.A., Fisher, S.L., Burn, P., Thiele, T.E., van Dijk, G., Baskin, D.G., Schwartz, M.W., 1997. Melanocortin receptors in leptin effects. Nature 390, 349.

Seidell, J.C., Flegal, K.M., 1997. Assessing obesity: classification and epidemiology. Br. Med. Bull. 53, 238–252.

Shibasaki, T., Yamauchi, N., Kato, Y., Masuda, A., Imaki, T., Hotta, M., Demura, H., Oono, H., Ling, N., Shizume, K., 1988. Involvement of corticotropin-releasing factor in restraint stress-induced anorxia and reversion of the anorexia by somatostatin in the rat. Life Sci. 43, 1103–1110.

Shimizu, N., Oomura, Y., Kai, Y., 1989. Stress-induced anorexia in rats mediated by serotonergic mechanisms in the hypothalamus. Physiol. Behav. 46, 835–841.

Skuladottir, G.V., Jonsson, L., Skarphedinsson, J.O., Mutulis, F., Muceniece, R., Raine, A., Mutule, I., Helgason, J., Prusis, P., Wikberg, J.E., Schiöth, H.B., 1999. Long term orexigenic effect of a novel melanocortin 4 receptor selective antagonist. Br. J. Pharmacol. 126, 27–34.

Slochower, J., 1976. Emotional labeling and overeating in obese and normal-weight individuals. Psychosom. Med. 38, 131–139.

Troop, N.A., Treasure, J.L., 1997. Psychosocial factors in the onset of eating disorders: responses to life-events and difficulties. Br. J. Med. Psychol. 70, 373–385.

Vaisse, C., Clement, K., Guy-Grand, B., Froguel, P., 1998. A frameshift mutation in human MC_4R is associated with a dominant form of obesity. Nat. Genet. 20, 113–114.

Vergoni, A.V., Poggioli, R., Bertolini, A., 1986. Corticotropin inhibits food intake in rats. Neuropeptides 7, 153–158.

Vergoni, A.V., Poggioli, R., Marrama, D., Bertolini, A., 1990. Inhibition of feeding by ACTH-(1–24): behavioral and pharmacological aspects. Eur. J. Pharmacol. 179, 347–355.

Vergoni, A.V., Bertolini, A., Wikberg, J.E., Schiöth, H.B., 1998. Differential influence of a selective melanocortin MC_4 receptor antagonist on melanocortin-induced behavioral effects in rats. Eur. J. Pharmacol. 362, 95–101.

Vergoni, A.V., Bertolini, A., Wikberg, J.E.S., Schiöth, H.B., 1999a. Selective melanocortin MC_4 receptor blockage reduces immobilization stress-induced anorexia in rats. Eur. J. Pharmacol. 369, 11–15.

Vergoni, A.V., Bertolini, A., Wikberg, J.E.S., Schiöth, H.B., 1999b. Corticotropin-releasing factor (CRF)-induced anorexia is not influenced by a melanocortin 4 receptor blockage. Peptides 20, 509–513.

Vergoni, A.V., Bertolini, A., Guidetti, G., Karefilakis, V., Filaferro, M., Wikberg, J.E.S., Schioth, H.B., 2000. Chronic melanocortin 4 receptor blockage causes obesity without influence on sexual behavior in male rats. J. Endocrinol., in press.

Wessells, H., Fuciarelli, K., Hansen, J., Hadley, M.E., Hruby, V.J., Dorr, R., Levine, N., 1998. Synthetic melanotropic peptide initiates erections in men with psychogenic erectile dysfunction: double-blind placebo controlled crossover study. J. Urol. 160, 389–393.

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

Yeo, G.S., Farooqi, I.S., Aminian, S., Halsall, D.J., Stanhope, R.G., O'Rahilly, S., 1998. A frameshift mutation in MC_4R associated with dominantly inherited human obesity. Nat. Genet. 20, 111–112.