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Review

Melanocortin MC_4 receptor expression sites and local function

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ABSTRACT

The melanocortin MC_4 receptor plays an important role in energy metabolism, but also affects blood pressure, heart rate and erectile function. Localization of the receptors that fulfill these distinct roles is only partially known. Mapping of the melanocortin MC_4 receptor has been stymied by the absence of a functional antibody. Several groups have examined mRNA expression of the melanocortin MC_4 receptor in the rodent brain and transgenic approaches have also been utilized to visualize melanocortin MC_4 receptor expression sites within the brain. Ligand expression and binding studies have provided additional information on the areas of the brain where this elusive receptor is functionally expressed. Finally, microinjection of melanocortin MC_4 receptor ligands in specific nuclei has further served to elucidate the function of melanocortin MC_4 receptors in these nuclei. These combined approaches have helped link the anatomy and function of this receptor, such as the role of paraventricular hypothalamic nucleus melanocortin MC_4 receptor in the regulation of food intake. Intriguingly, however, numerous expression-sites have been identified that have not been linked to a specific receptor function such as those along the optic tract and olfactory tubercle. Further research is needed to clarify the function of the melanocortin MC_4 receptor at these sites.

Contents

1. Introduction

The melanocortin receptor family consists of 5 G protein-coupled receptors, named the melanocortin MC_1 receptor through melanocortin MC_5 receptor. These family members are expressed

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throughout the body, including in skin melanocytes (melanocortin MC_1 receptor), the pituitary (melanocortin MC_2 receptor), the central nervous system (melanocortin MC_3 and MC_4 receptors) and in peripheral tissues (melanocortin MC_5 receptor). Determining which tissue types express specific melanocortin receptor family members is complicated by a lack of high affinity antibodies. Yet early studies with radioactively labeled ligands alluded to the presence of melanocortin receptors before they were discovered.

Melanocortin receptors are stimulated by melanocortins which are derived from posttranslational processing of the pre-prohormone pro-opiomelanocortin. These endogenous agonists include adrenocorticotrophin and the α-, β- and γ-melanocyte-stimulating hormones. The melanocortin receptors are antagonized by endogenous agouti and agouti-related protein. In addition to these endogenous ligands, numerous synthetic melanocortin receptor ligands have been produced. Commonly used synthetic ligands include the agonists Ac-Ser-Tyr-Ser-Nle-Glu-His-DPhe-Arg-Trp-Gly-Lys-Pro-Val-NH(2) (NDP-melanocyte-stimulating hormone), and melanotan-II and the antagonist SHU9119. Affinity for these and for the natural ligands varies among the melanocortin receptors. For the melanocortin MC_4 receptor, potency of some of the more commonly used ligands is as follows: melanotan-II>NDP-melanocyte-stimulating hormone≫adrenocorticotrophin>α-melanocyte-stimulating hormone≫γ-melanocyte-stimulating hormone (Oosterom et al., 1999; Adan et al., 1999). For the melanocortin MC_3 receptor, which is endogenously stimulated by β-melanocyte-stimulating hormone, ligand affinity is: NDP-melanocyte-stimulating hormone = melanotan-II≫β-melanocyte-stimulating hormone>γ-melanocyte-stimulating hormone>α-melanocyte-stimulating hormone>adrenocorticotrophin (Oosterom et al., 1999; Adan et al., 1999). Thus, among endogenous ligands, γ-melanocyte-stimulating hormone is more specific for the melanocortin MC_3 receptor, while affinities for α- and β-melanocyte-stimulating hormone are similar for both receptors. Frequent use of α-melanocyte-stimulating hormone in studies of the brain does not revealed the identity of the melanocortin receptor responsible for its actions.

Microinjection of ligands into specific nuclei of the brain has aided the elucidation of regional functions of melanocortin receptors and will be discussed in this review. Effects of intracerebroventricular injections of melanocortin receptor ligands cannot be attributed to single nuclei and are therefore outside the scope of this review.

2. Expression of the melanocortin MC_4 receptor in rodents

Melanocortin MC_4 receptor knockout mice and rats exhibit increased longitudinal growth and severe obesity, which is more severe in female mice (Huszar et al., 1997; van Boxtel et al., 2010). This obesity stems not only from overeating, but also from an enhanced caloric efficiency, as pair-feeding experiments in knockout mice have revealed (Ste Marie et al., 2000). Male knockout mice develop hyperglycemia by 3 months of age, and both sexes display elevated leptin and insulin levels (Huszar et al., 1997; Tallam et al., 2006). But loss of melanocortin MC_4 receptors also renders mice more sensitive to stress-related hypophagia (De Souza et al., 2000). In addition, these mice are not hypertensive and exhibit a lower basal heart rate (Tallam et al., 2005), and renal function and salt-sensitivity are unaltered in knockout mice (Tallam et al., 2006).

Expression of the melanocortin MC_4 receptor mRNA in the mouse brain was first demonstrated by Gantz and colleagues in 1993 (Gantz et al., 1993), and was explored in rats soon after by Mountjoy in the Cone laboratory (Mountjoy et al., 1994). A series of mRNA expression studies (Kishi et al., 2003), and the generation of transgenic animals expressing melanocortin MC4 receptor reporter genes (Liu et al., 2003; Daniel et al., 2005) have elucidated the sites at which melanocortin MC_4 receptor mRNA is transcribed in rodents. However, efforts to characterize expression of this receptor have been hampered by the fact that raising a specific antibody against a rodent melanocortin MC_4 receptor has proven difficult (van der Ploeg et al., 2002).

2.1. Forebrain

2.1.1. The Cortex

In rats as well as mice, melanocortin MC_4 receptor mRNA has been found throughout the cortex, most densely in layer 5 (Kishi et al., 2003; Liu et al., 2003). Weak or somewhat stronger expression has been observed in the auditory areas, orbital area and ventral temporal associated areas was seen, excluding the visual and somato sensory regions of mouse, but not rat cortices (Gantz et al., 1993; Mountjoy et al., 1994; Kishi et al., 2003; Hsu et al., 2005). A functional role for the melanocortin MC_4 receptor in auditory processes has not been established thus far. Several areas that are important for olfaction display a strong melanocortin MC_4 receptor signal. Within the olfactory cortex the olfactory tubercle displayed the strongest RNA signal more so than the piriform cortex, indicating a significant role for the melanocortin MC_4 receptor in the interpretation of olfaction (Kishi et al., 2003). In addition, some, but not all, *in situ* studies of the rat have shown expression of melanocortin MC_4 receptor mRNA in the taenia tecta (Mountjoy et al., 1994; Kishi et al., 2003).

2.1.2. The hippocampus

The hippocampus is the classical memory center of the brain.

Gantz and colleagues found high levels of melanocortin MC_4 receptor mRNA in the hippocampi of mice (Gantz et al., 1993), which was confirmed by Liu et al. (2003). In mouse dentate gyrus, CA1 and CA2—but not in CA3 or CA4—the labeling was extensive; however in rats the signal strength was only moderate to strong (Mountjoy et al., 1994) or even weak (Kishi et al., 2003) in these areas. Expression of melanocortin MC_4 receptor in these brain areas suggests a possible role for the melanocortin MC_4 receptor in memory. In the entorhinal and ventral portions of the subiculum, mRNA signal for the melanocortin MC_4 receptor was strong in some studies (Mountjoy et al., 1994), but weak in others (Kishi et al., 2003). Within the hippocampus, the ventral subiculum has been implicated in the regulation of the hypothalamic-pituitary-adrenal axis. This axis has a direct link to the melanocortin MC_4 receptor as first proposed by Lowry (2002).

To date, only a few studies have directly studied the effects of the melanocortin MC_4 receptor on memory. In rats, positive effects on memory have been observed after injection of α-melanocyte-stimulating hormone into the hippocampal CA1 region following injection of an inflammatory agent; however, no effects on fear memory were observed in response to α-melanocyte-stimulating hormone treatment alone (Gonzalez et al., 2009). Peripheral injection of adrenocorticotrophin has been shown to have strong positive effects on memory, in both humans and rodents (Miller et al., 1974). It is important to note, however, that melanocortin MC_3 receptor mRNA is also expressed weakly in the CA1–3 regions of the hippocampus (Roselli-Rehfuss et al., 1993). This makes it difficult to distinguish whether the effects of these melanocortins on memory are mediated by the melanocortin MC_3 or MC_4 receptor.

One peculiar effect of hippocampal lesions is that they have been shown to abolish α-melanocyte-stimulating hormone-induced stretching and yawning (Bertolini et al., 2009). Whether this abolishment is due to a direct effect on the hippocampus or because of the damaging nature of a lesion on fiber tracts in the surrounding area such as those that initiate at the septum is unknown.

2.1.3. The amygdala

The amygdala has been implicated in feeding behavior and weight gain through lesion studies of the medial amygdale, as reviewed in (King, 2006).

The medial amygdala of the rat is rich in melanocortin MC_4 receptor mRNA expression (Mountjoy et al., 1994; Kishi et al., 2003; Liu et al., 2003), more so than the central or basolateral nuclei which show moderate or no expression in their various subregions. The interstitial nucleus of the posterior limb of the anterior commissure also showed a prominent signal in one study (Kishi et al., 2003), and only one study found high expression in the nucleus of the lateral olfactory tract (Liu et al., 2003).

Support for functional melanocortin MC_4 receptor expression in the amgdala of rats comes from studies by Boghossian and colleagues. They found that injection of melanotan-II into the central bed nucleus of the amygdala reduces high-fat food intake, whilst having no effect on chow intake in rats on a 2-choice diet (Boghossian et al., 2010). Conversely, in the same study inhibition of melanocortin MC_3 and MC_4 receptors induced increased intake of the high-fat diet only. These data provide evidence for a melanocortin-driven fat-specific taste-aversion center in the amygdala.

2.1.4. The septal region

The septal nucleus connects the hypothalamus and the amygdala, and has strong reciprocal connections to the hippocampus. It has been associated with reward pathways and also long term memory potentiation.

The septal region of rats shows moderate to very strong melanocortin MC_4 receptor mRNA expression and the intermediate and the medial portions of the lateral septal nucleus as well as the circumventricular subfornical organ show the highest expression levels in rats (Mountjoy et al., 1994; Kishi et al., 2003), but the signal intensity in the septohippocampal nucleus varies between studies. In the lateral septal region, α-melanocyte-stimulating hormone-containing fibers are apparent to a moderate degree (Jacobowitz and O'Donohue, 1978), but moderate melanocortin MC_3 receptor mRNA expression was also found in the intermediate part of the lateral septum which could also account for α-melanocyte-stimulating hormone binding (Roselli-Rehfuss et al., 1993). In mice, however, only the dorsal and intermediate portions of the lateral septal nucleus contain a high number of melanocortin MC_4 receptor-positive cells, whereas the medial portion of this reward-related center was shown to have only a few receptor-positive cells (Liu et al., 2003).

Moderate levels of melanocortin MC_4 receptor mRNA were found throughout the subdivisions of the bed nucleus of the stria terminalis in rats (Mountjoy et al., 1994; Kishi et al., 2003), with highest expression in the principal nucleus (Mountjoy et al., 1994), and no expression in the supracapsular and subventricular subnuclei (Kishi et al., 2003). The stria terminalis is also exposed to a dense network of α-MSH-positive fibers (Jacobowitz and O'Donohue, 1978). Furthermore, only weak melanocortin MC_3 receptor mRNA signal was found in the dorsomedial and anterolateral portions of the bed of the nucleus stria terminalis (Roselli-Rehfuss et al., 1993). These combined findings argue for in favor of melanocortin MC_4 receptor-mediated actions of melanocortins at the bed nucleus of the stria terminals. This sexually dimorphic bed nucleus is not only associated with gender identity in humans (Kruijver et al., 2000) and reproduction, but also with reward and food intake (King, 2006) of which the latter two pertain to the melancortin MC_4 receptor.

2.1.5. The corpus striatum

The corpus striatum is rich in dopaminergic neurons, receives sensorimotor-related information, and plays a role in reward, decision making and feeding (Voorn et al., 2004).

Within the striatal zone, the fundus of the striatum and the caudate putamen show the greatest melanocortin MC_4 receptor labeling, followed by the nucleus accumbens, and less labeling is found in the magnocellular preoptic nucleus, substantia inomiata and ventral pallidum in rats (Mountjoy et al., 1994; Kishi et al., 2003; Hsu et al., 2005). Accordingly moderate number of α-melanocyte-stimulating hormone containing fibers were found in the stria terminalis, nucleus accumbens, and preoptic area, the existence of α-melanocyte-stimulating hormone-positive fibers in the caudate putamen is not mentioned, however (Jacobowitz and O'Donohue, 1978), nor is NPD-melanocyte-stimulating hormone found to bind extensively here (Lichtensteiger et al., 1996).

In mice only the shell of the nucleus accumbens, ventral pallidum, substantia inominata and a few cells in the caudate putamen and medial globus pallidus were labeled (Liu et al., 2003). The nucleus accumbens in the corpus striatum is an important anatomical structure in the reward pathway. A recent study in rats demonstrated that SHU9119 injected into the nucleus accumbens blocked cocaine-induced locomotor activity and reward behavior (Hsu et al., 2005). Furthermore, the authors found that melanocortin MC_4 receptor null mice also do not exhibit the characteristic locomotor response to cocaine administration; an additional indication of a functional role for melanocortin MC_4 receptors in the nucleus accumbens area.

2.1.6. The thalamus

The thalamus is one of the few regions of the brain in which only a small number of nuclei are positive for melanocortin MC4 receptor mRNA (Gantz et al., 1993). In both mouse and rat thalamus weak signal is seen in the nucleus reuniens and lateral habenular nucleus, weak to moderate signal in the zona incerta and the magnocellular portion of the subparafascicular nucleus and a stronger signal in the suprageniculate nucleus (Mountjoy et al., 1994; Kishi et al., 2003). The thalamus is rich in α-melanocyte-stimulating hormone-containing fibers but no evidence of melanocortin receptor function in this relay station of the brain has been found to date.

2.1.7. The hypothalamus

The hypothalamus consists of a heterogenous population of nuclei that are responsible for maintaining whole body homeostasis. Melanocortin MC_4 receptor expression within the hypothalamus is widespread, and has been extensively studied. In rats expression of this receptor is highest in the supraoptic nucleus and the anteroventral periventricular nucleus, but strong labeling is also seen in the preoptic nucleus, in the subdivisions of the paraventricular nucleus and to a lesser extent in the arcuate nucleus (Mountjoy et al., 1994; Kishi et al., 2003). Mice show an almost identical pattern for melanocortin MC_4 receptor expression, with the exception of the supraoptic nucleus (Liu et al., 2003). Dense fibers containing α-melanocyte-stimulating hormone are also found in the hypothalamic areas of rats that express melanocortin MC_4 receptor mRNA (Jacobowitz and O'Donohue, 1978). The melanocortin MC_4 receptors in the anteroventral periventricular nucleus and in the paraventricular nucleus are thought to mediate the neuroendocrine effects of the receptor, as well as regulating food intake. The necessity of such high expression levels of melanocortin MC_4 receptor in the preoptic area is unclear. However, a link between sight and melanocortins was established in visual memory experiments performed on humans and rats injected with melanocortins in the periphery (Miller et al., 1974). Both visual memory and attention was also enhanced in the volunteers, while extinction of memory was inhibited in rats (de Wied, 1966). Unfortunately, it cannot be determined from these experiments whether these effects were exclusively caused by melanocortin MC_4 receptors.

Some studies have found strong for melanocortin MC_4 receptor mRNA signal in the suprachiasmatic nucleus of rats (Mountjoy et al., 1994), but others failed to find signal in the circadian rhythm nucleus

in rats and mice (Liu et al., 2003; Kishi et al., 2003). Whether differences in this small oscillating nucleus (Kalsbeek et al., 2006) are due to interspecies or experimental differences, such as time of death, is unknown.

Beyond the periventricular area, the medial hypothalamus is also rich in melanocortin MC_4 receptor mRNA. Expression is most obvious in the medial preoptic area of rats, but not of mice, and in the anteroventral preoptic nucleus, the ventrolateral portion of the ventromedial nucleus of the hypothalamus, the anterior portion of the dorsomedial nucleus of the hypothalamus and in some studies, but not all, in the tuberomammilary body (Gantz et al., 1993; Mountjoy et al., 1994; Kishi et al., 2003; Liu et al., 2003). Again, α-MSH containing fibers were found in these melanocortin MC_4 receptor expressing areas (Jacobowitz and O'Donohue, 1978). The lateral zone of the hypothalamus shows weak to moderate melanocortin MC_4 receptor mRNA expression in the lateral preoptic area and in the lateral hypothalamic area and both these areas have abundant α-melanocyte-stimulating hormone innervation (Jacobowitz and O'Donohue, 1978; Mountjoy et al., 1994; Kishi et al., 2003). While the melanocortin MC_3 receptor is also expressed throughout this area of the brain, although at seemingly lower levels, the family members overlap to a great extent in areas including the ventromedial nucleus of the hypothalamus, arcuate nucleus, anteroventral preoptic nucleus, medial preoptic area, anterior hypothalamic area and the lateral hypothalamic area (Mountjoy et al., 1994; Roselli-Rehfuss et al., 1993). This overlap in expression makes it exceedingly difficult to distinguish the functions of the 2 receptors in the hypothalamic area.

Early studies showed that administration of α-melanocyte-stimulating hormone or its synthetic analogue melanotan-II increased stretching, yawning and grooming behavior as well as locomotor activity in rodents (Bressers et al., 1995; Adan et al., 1999). Injection of α-melanocyte-stimulating hormone in the paraventricular, dorsomedial or ventromedial nucleus of hypothalamus, or in the anterior hypothalamus significantly increased grooming, stretching, yawning and penile erection in rats. All of these but the erectile effect can be blocked in a dose-dependent fashion by pretreatment with a melanocortin antagonist (Argiolas et al., 2000).

Melanocortin MC_4 receptors in the paraventricular nucleus play an important role in regulating energy balance. Unilateral stereotaxic microinjection of melanotan-II into the paraventricular nucleus potently increases food intake, and can prevent the orexigenic effects of neuropeptide Y injected at the same site (Cowley et al., 1999). Furthermore, the central effects of intraventricular injection of melanotan-II on oxygen consumption can also be recapitulated by microinjection with the same substance in the paraventricular nucleus of mice (Cowley et al., 1999). Antagonizing the receptor by viral expression of agouti-related protein in the paraventricular nucleus of rats leads to increased food intake and body weight within 7 days of bilateral injection (Kas et al., 2004). Knockdown of the receptor itself by bilateral injection of shRNA against it in the paraventricular nucleus leads to an increase in high-fat diet intake and a subsequent increase in body weight in rats. Reduced receptor expression does not, however, affect food intake in animals on a chow diet (Garza et al., 2008). That the paraventricular nucleus is not the only nucleus mediating melanocortin-driven food intake was shown by both acute injections of agonist and antagonist as well as viral-mediated long term overexpression of agouti-related protein in various nuclei. Aside from the paraventricular nucleus, injection into the dorsal medial hypothalamus, medial preoptic area and to a lesser extend the anterior hypothalamic area and amygdala, but not the arcuate nucleus or the ventromedial nucleus significantly influences food intake (Kim et al., 2000; Kas et al., 2004).

Evidence for separate functions of melanocortin MC_4 receptors at different locations in energy metabolism was elegantly demonstrated by N. Balthasar and her colleagues. By creating a transgenic mouse that only expressed melanocortin MC_4 receptor in the paraventricular, the supraoptic, the posterior hypothalamic, and the lateral olfactory tract nucleus as well as the ventromedial and lateral hypothalamus, the amgdala, the medial preoptic nucleus and other areas, melanocortin MC_4 receptor-regulated food intake was completed rescued compared to the complete melanocortin MC_4 receptor knock out mouse (Balthasar et al., 2005). The reduction in energy expenditure that is seen in the absence of the receptor was still intact in this mouse model, indicating that melanocortin MC_4 receptors elsewhere play a role in regulating it.

2.2. The Brainstem

The brainstem regulates vital functions such as respiration, cardiac function and motor functions. The brainstem harbours some of the strongest melanocortin MC_4 receptor mRNA labeling within the brain. Melanocortin MC_4 receptor-positive cells can be found along the optic tract, showing maximum intensity in the optic layer and in the nucleus of the optic tract, and moderate intensity in the intermediate gray layer and the deep gray layer of the superior colliculus of both mice and rats (Kishi et al., 2003; Mountjoy et al., 1994). These findings suggest a role for the melanocortin MC_4 receptor in processing of visual information in the brainstem (Mountjoy et al., 1994) though little research has been published in this arena.

A faint melanocortin MC_4 receptor mRNA signal was observed in the nucleus of the lateral lemniscus but the function of melanocortin MC_4 receptors here is unclear. A faint signal is also visible in the pars medialis of the nucleus of the solitary tract and a faint to moderate signal in the parabrachial nucleus of mice and rats (Mountjoy et al., 1994; Kishi et al., 2003). The gustatory processing that takes place in the parabrachial nucleus may explain some of the melanocortin MC_4 receptor on high-fat food preference.

A second region of very high intensity melanocortin MC_4 receptor mRNA expression is found in the dorsal motor nucleus of the vagus nerve (Gantz et al., 1993; Mountjoy et al., 1994). The dorsal motor nucleus of the vagus nerve is closely connected to the vagus nerve that sends and receives parasympathetic innervation to and from the gastrointestinal tract and abdomen as well as the liver. Administration of melanocortin MC_4 receptor ligands into the fourth ventricle has a profound effect on food intake and body weight (Grill et al., 1998), and provided the first indication of a role for brainstem melanocortin MC_4 receptors in the maintenance of energy homeostasis. Further pharmacological studies of the densely labeled dorsal motor nucleus of the vagus nerve have revealed significant functional effects of the melanocortin MC_4 receptor here (Williams et al., 2000).

Other labeled sites within the motor region that show moderate or low melanocortin MC_4 receptor mRNA expression are the ventral division of the nucleus ambiguus and the compact and reticular portions of the substantia nigra. The nucleus ambiguus houses the preganglionic parasympathetic vagal neurons that innervate the heart. The effects of the melanocortin MC4 receptor on the cardiovascular system may be at least partially mediated at this site. The substantia nigra is involved in addiction, motivation and reward, as is the ventral tegmental area. Weak melanocortin MC_4 receptor mRNA staining was found in the ventral tegmental area and the inferior salivatory nucleus of the rat (Mountjoy et al., 1994).

In addition, moderate to strong labeling was found in the red nucleus and moderate to weak labeling in several regions of the reticular core, the raphé and the reticular formation of mice and rats (Mountjoy et al., 1994; Kishi et al., 2003). What the function of the melanocortin MC_4 receptor at the red nucleus is, is not evident. However, the abundance of α-melanocyte-stimulating hormone-positive fibers at this nucleus add to the prerequisites of a functional melanocortin system at this site. In contrast, only few α-melanocyte-stimulating hormone-positive fibers were visible in the reticular

formation (Jacobowitz and O'Donohue, 1978). Even so, one might speculate that melanocortin MC_4 receptor-driven effects on cardiac function may partially be mediated at this site.

2.3. The spinal cord

In lieu of a functional antibody, binding of labeled agonist in combination with Northern blot analysis alluded to the presence of melanocortin MC_4 receptors on the spinal cord (van der Kraan et al., 1999), confirming earlier findings from *in situ* hybridizations (Mountjoy et al., 1994). Both groups found that the melanocortin MC_4 receptor is expressed at the border between the marginal zone and the substantia gelatinosa of the spinal cord of rats. A third study also found strong expression of melanocortin MC_4 receptor mRNA in lamina 1 and 2 of the dorsal horn, and weak expression in the ventral horn (Kishi et al., 2003). This expression pattern extends along the thoracic cord, and increases in signal strength through the caudal levels.

Spinal cord and peripheral nervous system melanocortin MC_4 receptor has been implicated in conveying the neurotrophic effects of melanocortins (van der Kraan et al., 1999).

2.4. Peripheral melanocortin MC_4 receptor expression

The melanocortin MC_3 receptor and melanocortin MC_4 receptor have been dubbed the brain melanocortin receptors, and early reports found no mRNA expression of melanocortin MC_4 receptor in the periphery. Visible levels of melanocortin MC_4 receptor mRNA were absent in the mouse placenta (Gantz et al., 1993), the rat stomach, liver, kidney, adrenals, heart and spleen (Mountjoy et al., 1994). Furthermore, the lack of effects of melanocortin MC_4 receptor antibodies when injected in the periphery versus central injection argues in favor of the absence of functional melanocortin MC_4 receptors outside of the central nervous system (Peter et al., 2007).

However, later studies by the same authors, and using the same probe previously described, found expression of melanocortin MC_4 receptors in a wide range of fetal rat tissues (Mountjoy and Wild, 1998; Mountjoy et al., 2003). Organs such as the adrenal and kidney medullae, and the penis that are innervated by the sympathetic nervous system displayed the highest signal to noise ratios in the third trimester, but the cardiac, respiratory and muscoskeletal systems also show a burst of melanocortin MC_4 receptor mRNA expression. *In situ* hybridization did not detect melanocortin MC_4 receptor mRNA in adult animals in these tissues, but it is unclear whether this is due to its complete absence or due to low expression in these tissues (Mountjoy et al., 2003). Further studies confirming the peripheral expression patterns observed in rats embryos are needed to clarify this enigma. In mice, a study found melanocortin MC_4 receptor expression in the periphery by utilizing the *MC4R* promoter driven expression of green fluorescent protein. Here, a markedly different expression pattern was reported from that seen in the embryonic rat, including expression in the nodose ganglion, and in nerve fibers innervating the stomach, the portal hilus of the liver, and the duodenum (Gautron et al., 2010). Though this evidence could potentially provide a convenient explanation for the observed decrease in sympathetic tone in human patients with melanocortin MC_4 receptor mutations, further investigation is needed to firmly establish peripheral melanocortin MC_4 receptor expression.

3. Distribution of the melanocortin MC_4 receptor in humans

Mutations in the melanocortin MC_4 receptor are the single largest monogenetic contribution to obesity in the human population, accounting for 2–5% of cases (Vaisse et al., 2000; Farooqi et al., 2000). Childhood obesity due to melanocortin MC_4 receptor alterations is accompanied by elevation of blood glucose levels and hyperinsulinemia (Farooqi et al., 2003), as is seen in rodents lacking melanocortin MC_4 receptors. Melanocortin MC_4 receptor mutations in human patients that reduce receptor function also reduce blood pressure and heart rate, and are associated with a decreased prevalence of hypertension associated with mutations in melanocortin MC_4 receptor (Greenfield et al., 2009; Sayk et al., 2010). An inverse relationship between muscle sympathetic nerve activity and obesity has been observed in a small study, indicating that reduced sympathetic tone may be caused by melanocortin MC_4 receptor mutations (Sayk et al., 2010). Thus the reduced sympathetic tone of patients with melanocortin MC_4 receptor mutations helps protect them against obesity-induced hypertension.

Few studies mapping the expression of the melanocortin MC_4 receptor in the human body have been published. While examination of cDNA from twenty different human tissues only revealed expression of melanocortin MC_4 receptor in human pituitary extracts (Chhajlani, 1996), another study found melanocortin MC_4 receptor mRNA in a sensory corpuscle of one human penis (van der Ploeg et al., 2002). A Tucson trial of penile function in the presence of melanotan-II in 10 men with erectile dysfunction revealed beneficial effects of melanotan-II with regards to arousal and function. However, a negative side effect of melanotan-II revealed in this study was a high incidence of severe nausea, limiting its clinical usefulness (Wessells et al., 2000). The mechanism by which melanotan-II improves erectile function is unknown and it is unclear whether local or central melanocortin receptors are responsible.

Aside from the two expression reports, the distribution of melanocortin MC_4 receptor expression in humans is a virtually untouched field.

4. Discussion

The diversity of melanocortin MC_4 receptor expression seems indicative of a multitude of biological functions. While most commonly considered a regulator of energy homeostasis, the melanocortin MC_4 receptor is also expressed in sites that are not involved in energy balance. We can, for example only speculate as to what the physiological function of the receptor is within the optic tract or the hippocampus.

Absence of a functional antibody for the melanocortin MC_4 receptor is a significant roadblock when it comes to mapping the expression pattern of the receptor. The various functions of melanocortins in the brain and concomitant phenotypical alterations due to loss of one or both alleles in rodents or human patients suggest that the melanocortin MC_4 receptor is expressed at multiple brain sites. Information from ligand-binding studies, mRNA labeling and the effects of microinjection of ligands have helped to elucidate locations of functional expression of the melanocortin MC_4 receptor. Of these methods, each has its drawbacks, such as the similar affinity of several ligands for melanocortin MC_3 and MC_4 receptors, possible posttranslational measures affecting melanocortin MC_4 receptor expression, and non-specific pharmacological effects of microinjections of high concentrations of ligand.

Aside from these methodological limitations, the expression patterns and effects of the melanocortin MC_4 receptor that have been observed in rodents may not translate to the human situation. Our knowledge of human melanocortin receptor expression is still in its infancy despite the fact that melanocortins have been studied in humans since 1947 (Mason and Power, 1947).

The discrepancies in melanocortin MC_4 receptor expression patterns and expression intensity can be explained by a large number of variables. In addition to the variation that is expected between groups, species and methodologies, a number of environmental factors may also influence expression of the melanocortin MC_4 receptor. Factors influencing melanocortin MC_4 receptor expression may include maternal diet (Gout et al., 2010), fasting state (Germano et al., 2008), thyroid function (Decherf et al., 2010), time of day can be of importance in some species, such as the Siberian hamster (Ellis

et al., 2008), or even cocaine addiction, which has been shown to reduce melanocortin MC_4 receptor expression (Hsu et al., 2005). Possible regulation of melanocortin MC_4 receptor expression in some, but not other regions justifies further research on the local function of the melanocortin MC_4 receptor, and may even expand its therapeutic potential, enabling melanocortin agents to be used that treat erectile dysfunction or obesity without side effects such as nausea.

Conflict of interest

The author declares no conflict of interest.

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References

Adan, R.A., Szklarczyk, A.W., Oosterom, J., Brakkee, J.H., Nijenhuis, W.A., Schaaper, W.M., Meloen, R.H., Gispen, W.H., 1999. Characterization of melanocortin receptor ligands on cloned brain melanocortin receptors and on grooming behavior in the rat. Eur. J. Pharmacol. 378, 249–258.

Argiolas, A., Melis, M.R., Murgia, S., Schioth, H.B., 2000. Brain Res. Bull. 51, 425–431.

Balthasar, N., Dalgaard, L.T., Lee, C.E., Yu, J., Funahashi, H., Williams, T., Ferreira, M., Tang, V., McGovern, R.A., Kenny, C.D., Christiansen, L.M., Edelstein, E., Choi, B., Boss, O., Aschkenasi, C., Zhang, C.Y., Mountjoy, K., Kishi, T., Elmquist, J.K., Lowell, B.B., 2005. Divergence of melanocortin pathways in the control of food intake and energy expenditure. Cell 123, 493–505.

Bertolini, A., Tacchi, R., Vergoni, A.V., 2009. Brain effects of melanocortins. Pharmacol. Res. 59, 13–47.

Boghossian, S., Park, M., York, D.A., 2010. Melanocortin activity in the amygdala controls appetite for dietary fat. Am. J. Physiol. Regul. Integr. Comp. Physiol. 298, R385–R393.

Bressers, W.M., Kruk, M.R., Van Erp, A.M., Willekens-Bramer, D.C., Haccou, P., Meelis, E., 1995. A time-structured analysis of hypothalamically induced increases in self-grooming and activity in the rat. Behav. Neurosci. 109, 1158–1171.

Chhajlani, V., 1996. Distribution of cDNA for melanocortin receptor subtypes in human tissues. Biochem. Mol. Biol. Int. 38, 73–80.

Cowley, M.A., Pronchuk, N., Fan, W., Dinulescu, D.M., Colmers, W.F., Cone, R.D., 1999. Integration of NPY, AGRP, and melanocortin signals in the hypothalamic paraventricular nucleus: evidence of a cellular basis for the adipostat. Neuron 24, 155–163.

Daniel, P.B., Fernando, C., Wu, C.S., Marnane, R., Broadhurst, R., Mountjoy, K.G., 2005. 1 kb of 5′ flanking sequence from mouse MC4R gene is sufficient for tissue specific expression in a transgenic mouse. Mol. Cell. Endocrinol. 239, 63–71.

De Souza, J., Butler, A.A., Cone, R.D., 2000. Disproportionate inhibition of feeding in A(y) mice by certain stressors: a cautionary note. Neuroendocrinology 72, 126–132.

De Wied, D., 1966. Inhibitory effect of ACTH and related peptides on extinction of conditioned avoidance behavior in rats. Proc. Soc. Exp. Biol. Med. 122, 28–32.

Decherf, S., Seugnet, I., Kouidhi, S., Lopez-Juarez, A., Clerget-Froidevaux, M.S., Demeneix, B.A., 2010. Thyroid hormone exerts negative feedback on hypothalamic type 4 melanocortin receptor expression. Proc. Natl. Acad. Sci. U. S. A. 107, 4471–4476.

Ellis, C., Moar, K.M., Logie, T.J., Ross, A.W., Morgan, P.J., Mercer, J.G., 2008. Diurnal profiles of hypothalamic energy balance gene expression with photoperiod manipulation in the Siberian hamster, Phodopus sungorus. Am. J. Physiol. Regul. Integr. Comp. Physiol. 294, R1148–R1153.

Farooqi, I.S., Yeo, G.S., Keogh, J.M., Aminian, S., Jebb, S.A., Butler, G., Cheetham, T., O'Rahilly, S., 2000. Dominant and recessive inheritance of morbid obesity associated with melanocortin 4 receptor deficiency. J. Clin. Invest. 106, 271–279.

Farooqi, I.S., Keogh, J.M., Yeo, G.S., Lank, E.J., Cheetham, T., O'Rahilly, S., 2003. Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. N. Engl. J. Med. 348, 1085–1095.

Gantz, I., Miwa, H., Konda, Y., Shimoto, Y., Tashiro, T., Watson, S.J., DelValle, J., Yamada, T., 1993. Molecular cloning, expression, and gene localization of a fourth melanocortin receptor. J. Biol. Chem. 268, 15174–15179.

Garza, J.C., Kim, C.S., Liu, J., Zhang, W., Lu, X.Y., 2008. Adeno-associated virus-mediated knockdown of melanocortin-4 receptor in the paraventricular nucleus of the hypothalamus promotes high-fat diet-induced hyperphagia and obesity. J. Endocrinol. 197, 471–482.

Gautron, L., Lee, C., Funahashi, H., Friedman, J., Lee, S., Elmquist, J., 2010. Melanocortin-4 receptor expression in a vago-vagal circuitry involved in postprandial functions. J. Comp. Neurol. 518, 6–24.

Germano, C.M., Castro, M., Rorato, R., Costa, D.B., Antunes-Rodrigues, J., Elias, C.F., Elias, L.L., 2008. Downregulation of melanocortin-4 receptor during refeeding and its modulation by adrenalectomy in rats. Horm. Metab. Res. 40, 842–847.

Gonzalez, P.V., Schioth, H.B., Lasaga, M., Scimonelli, T.N., 2009. Memory impairment induced by IL-1beta is reversed by alpha-MSH through central melanocortin-4 receptors. Brain Behav. Immun. 23, 817–822.

Gout, J., Sarafian, D., Mutel, E., Vigier, M., Rajas, F., Mithieux, G., Begeot, M., Naville, D., 2010. Metabolic and melanocortin gene expression alterations in male offspring of obese mice. Mol. Cell. Endocrinol. 319, 99–108.

Greenfield, J.R., Miller, J.W., Keogh, J.M., Henning, E., Satterwhite, J.H., Cameron, G.S., Astruc, B., Mayer, J.P., Brage, S., See, T.C., Lomas, D.J., O'Rahilly, S., Farooqi, I.S., 2009. Modulation of blood pressure by central melanocortinergic pathways. N. Engl. J. Med. 360, 44–52.

Grill, H.J., Ginsberg, A.B., Seeley, R.J., Kaplan, J.M., 1998. Brainstem application of melanocortin receptor ligands produces long-lasting effects on feeding and body weight. J. Neurosci. 18, 10128–10135.

Hsu, R., Taylor, J.R., Newton, S.S., Alvaro, J.D., Haile, C., Han, G., Hruby, V.J., Nestler, E.J., Duman, R.S., 2005. Blockade of melanocortin transmission inhibits cocaine reward. Eur. J. Neurosci. 21, 2233–2242.

Huszar, D., Lynch, C.A., Fairchild-Huntress, V., Dunmore, J.H., Fang, Q., Berkemeier, 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.

Jacobowitz, D.M., O'Donohue, T.L., 1978. alpha-Melanocyte stimulating hormone: immunohistochemical identification and mapping in neurons of rat brain. Proc. Natl. Acad. Sci. U. S. A. 75, 6300–6304.

Kalsbeek, A., Palm, I.F., La Fleur, S.E., Scheer, F.A., Perreau-Lenz, S., Ruiter, M., Kreier, F., Cailotto, C., Buijs, R.M., 2006. SCN outputs and the hypothalamic balance of life. J. Biol. Rhythms 21, 458–469.

Kas, M.J., Tiesjema, B., van Dijk, G., Garner, K.M., Barsh, G.S., ter Beek, O., Verhaagen, J., Adan, R.A., 2004. Induction of brain-region-specific forms of obesity by agouti. J. Neurosci. 24, 10176–10181.

Kim, S.M., Rossi, M., Abusnana, S., Sunter, D., Morgan, D.G.A., Small, C.J., Edwards, C.M.B., Heath, M.M., Stanley, S.A., Seal, L.J., Bhatti, J.R., Smith, D.M., Ghatei, M.A., Bloom, S.R., 2000. Hypothalamic localization of the feeding effects of agouti-related peptide and alpha-melanocyte-stimulating hormone. Diabetes 49, 177–182.

King, B.M., 2006. Amygdaloid lesion-induced obesity: relation to sexual behavior, olfaction, and the ventromedial hypothalamus. Am. J. Physiol. Regul. Integr. Comp. Physiol. 291, R1201–R1214.

Kishi, T., Aschkenasi, C.J., Lee, C.E., Mountjoy, K.G., Saper, C.B., Elmquist, J.K., 2003. Expression of melanocortin 4 receptor mRNA in the central nervous system of the rat. J. Comp. Neurol. 457, 213–235.

Kruijver, F.P., Zhou, J.N., Pool, C.W., Hofman, M.A., Gooren, L.J., Swaab, D.F., 2000. Male-to-female transsexuals have female neuron numbers in a limbic nucleus. J. Clin. Endocrinol. Metab. 85, 2034–2041.

Lichtensteiger, W., Hanimann, B., Siegrist, W., Eberle, A.N., 1996. Region- and stage-specific patterns of melanocortin receptor ontogeny in rat central nervous system, cranial nerve ganglia and sympathetic ganglia. Brain Res. Dev. Brain Res. 91, 93–110.

Liu, H., Kishi, T., Roseberry, A.G., Cai, X., Lee, C.E., Montez, J.M., Friedman, J.M., Elmquist, J.K., 2003. Transgenic mice expressing green fluorescent protein under the control of the melanocortin-4 receptor promoter. J. Neurosci. 23, 7143–7154.

Lowry, C.A., 2002. Functional subsets of serotonergic neurones: implications for control of the hypothalamic-pituitary-adrenal axis. J. Neuroendocrinol. 14, 911–923.

Mason, H.L., Power, M.H., 1947. Results of administration of anterior pituitary adrenocorticotropic hormone to a human subject. J. Clin. Endocrinol. Metab. 7, 458.

Miller, L.H., Kastin, A.J., Sandman, C.A., Fink, M., Van Veen, W.J., 1974. Polypeptide influences on attention, memory and anxiety in man. Pharmacol. Biochem. Behav. 2, 663–668.

Mountjoy, K.G., Wild, J.M., 1998. Melanocortin-4 receptor mRNA expression in the developing autonomic and central nervous systems. Brain Res. Dev. Brain Res. 107, 309–314.

Mountjoy, K.G., Mortrud, M.T., Low, M.J., Simerly, R.B., Cone, R.D., 1994. Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. Mol. Endocrinol. 8, 1298–1308.

Mountjoy, K.G., Jenny Wu, C.S., Dumont, L.M., Wild, J.M., 2003. Melanocortin-4 receptor messenger ribonucleic acid expression in rat cardiorespiratory, musculoskeletal, and integumentary systems. Endocrinology 144, 5488–5496.

Oosterom, J., Nijenhuis, W.A., Schaaper, W.M., Slootstra, J., Meloen, R.H., Gispen, W.H., Burbach, J.P., Adan, R.A., 1999. Conformation of the core sequence in melanocortin peptides directs selectivity for the melanocortin MC3 and MC4 receptors. J. Biol. Chem. 274, 16853–16860.

Peter, J.C., Nicholson, J.R., Heydet, D., Lecourt, A.C., Hoebeke, J., Hofbauer, K.G., 2007. Antibodies against the melanocortin-4 receptor act as inverse agonists in vitro and in vivo. Am. J. Physiol. Regul. Integr. Comp. Physiol. 292, R2151–R2158.

Roselli-Rehfuss, L., Mountjoy, K.G., Robbins, L.S., Mortrud, M.T., Low, M.J., Tatro, J.B., Entwistle, M.L., Simerly, R.B., Cone, R.D., 1993. Identification of a receptor for gamma melanotropin and other proopiomelanocortin peptides in the hypothalamus and limbic system. Proc. Natl. Acad. Sci. U. S. A. 90, 8856–8860.

Sayk, F., Heutling, D., Dodt, C., Iwen, K.A., Wellhoner, J.P., Scherag, S., Hinney, A., Hebebrand, J., Lehnert, H., 2010. Sympathetic function in human carriers of melanocortin-4 receptor gene mutations. J. Clin. Endocrinol. Metab. 95, 1998–2002.

Ste Marie, L., Miura, G.I., Marsh, D.J., Yagaloff, K., Palmiter, R.D., 2000. A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors. Proc. Natl. Acad. Sci. U. S. A. 97, 12339–12344.

Tallam, L.S., Stec, D.E., Willis, M.A., da Silva, A.A., Hall, J.E., 2005. Melanocortin-4 receptor-deficient mice are not hypertensive or salt-sensitive despite obesity, hyperinsulinemia, and hyperleptinemia. Hypertension 46, 326–332.

Tallam, L.S., da Silva, A.A., Hall, J.E., 2006. Melanocortin-4 receptor mediates chronic cardiovascular and metabolic actions of leptin. Hypertension 48, 58–64.

Vaisse, C., Clement, K., Durand, E., Hercberg, S., Guy-Grand, B., Froguel, P., 2000. Melanocortin-4 receptor mutations are a frequent and heterogeneous cause of morbid obesity. J. Clin. Invest. 106, 253–262.

Van Boxtel, R., Vroling, B., Toonen, P., Nijman, I.J., van Roekel, H., Verheul, M., Baakman, C., Guryev, V., Vriend, G., Cuppen, E., 2010. Systematic generation of in vivo G protein-coupled receptor mutants in the rat. Pharmacogenomics J. doi:10.1038/tpj.2010.4.

van der Kraan, M., Tatro, J.B., Entwistle, M.L., Brakkee, J.H., Burbach, J.P., Adan, R.A., Gispen, W.H., 1999. Expression of melanocortin receptors and pro-opiomelanocortin in the rat spinal cord in relation to neurotrophic effects of melanocortins. Brain Res. Mol. Brain Res. 63, 276–286.

van der Ploeg, L.H., Martin, W.J., Howard, A.D., Nargund, R.P., Austin, C.P., Guan, X., Drisko, J., Cashen, D., Sebhat, I., Patchett, A.A., Figueroa, D.J., DiLella, A.G., Connolly, B.M., Weinberg, D.H., Tan, C.P., Palyha, O.C., Pong, S.S., Macneil, T., Rosenblum, C., Vongs, A., Tang, R., Yu, H., Sailer, A.W., Fong, T.M., Huang, C., Tota, M.R., Chang, R.S., Stearns, R., Tamvakopoulos, C., Christ, G., Drazen, D.L., Spar, B.D., Nelson, R.J., Macintyre, D.E., 2002. A role for the melanocortin 4 receptor in sexual function. Proc. Natl. Acad. Sci. U. S. A. 99, 11381–11386.

Voorn, P., Vanderschuren, L.J., Groenewegen, H.J., Robbins, T.W., Pennartz, C.M., 2004. Putting a spin on the dorsal-ventral divide of the striatum. Trends Neurosci. 27, 468–474.

Wessells, H., Gralnek, D., Dorr, R., Hruby, V.J., Hadley, M.E., Levine, N., 2000. Effect of an alpha-melanocyte stimulating hormone analog on penile erection and sexual desire in men with organic erectile dysfunction. Urology 56, 641–646.

Williams, D.L., Kaplan, J.M., Grill, H.J., 2000. The role of the dorsal vagal complex and the vagus nerve in feeding effects of melanocortin-3/4 receptor stimulation. Endocrinology 141, 1332–1337.