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Review

Melanocortins and cardiovascular regulation

Dirk H.G. Versteeg *, Patricia Van Bergen, Roger A.H. Adan, Dick J. De Wildt

Department of Medical Pharmacology, Rudolf Magnus Institute for Neurosciences, Utrecht University, Universiteitsweg 100, 3584 CG Utrecht, Netherlands

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Abstract

The melanocortins form a family of pro-opiomelanocortin-derived peptides that have the melanocyte-stimulating hormone (MSH) core sequence, His–Phe–Arg–Trp, in common. Melanocortins have been described as having a variety of cardiovascular effects. We review here what is known about the sites and mechanisms of action of the melanocortins with respect to their effects on cardiovascular function, with special attention to the effects of the γ-melanocyte-stimulating hormones (γ-MSHs). This is done in the context of present knowledge about agonist selectivity and localisation of the five melanocortin receptor subtypes cloned so far. γ_2-MSH, its des-Gly^{12} analog (= γ_1-MSH) and Lys-γ_2-MSH are 5–10 times more potent than adrenocorticotropic hormone-(4–10) (ACTH-(4–10)) to induce a pressor and tachycardiac effect following intravenous administration. The Arg–Phe sequence near the C-terminal seems to be important for full in vivo intrinsic activity. Related peptides with a C-terminal extension with (γ_3-MSH) or without the Arg–Phe sequence (α-MSH, as well as the potent α-MSH analog, [Nle^4,D-Phe^7]α-MSH), are, however, devoid of these effects. In contrast, ACTH-(1–24) has a depressor effect combined with a tachycardiac effect, effects which are not dependent on the presence of the adrenals. Although the melanocortin MC_3 receptor is the only melanocortin receptor subtype for which γ_2-MSH is selective, in vivo and in vitro structure–activity data indicate that it is not via this receptor that this peptide and related peptides exert either their pressor and tachycardiac effects or their extra- and intracranial blood flow increasing effect. We review evidence that the pressor and tachycardiac effects of the γ-MSHs are due to an increase of sympathetic outflow to the vasculature and the heart, secondary to activation of centrally located receptors. These receptors are most likely localised in the anteroventral third ventricle (AV3V) region, a brain region situated outside the blood-brain barrier, and to which circulating peptides have access. These receptors might be melanocortin receptors of a subtype yet to be identified. Alternatively, they might be related to other receptors for which peptides with a C-terminal Arg–Phe sequence have affinity, such as the neuropeptide FF receptor and the recently discovered FMRFamide receptor. Melanocortin MC_4 receptors and still unidentified receptors are part of the circuitry in the medulla oblongata which is involved in the depressor and bradycardiac effect of the melanocortins, probably via interference with autonomic outflow. Regarding the effects of the γ-MSHs on cortical cerebral blood flow, it is not yet clear whether they involve activation of the sympathetic nervous system or activation of melanocortin receptors located on the cerebral vasculature. The depressor effect observed following intravenous administration of ACTH-(1–24) is thought to be due to activation of melanocortin MC_2 receptors whose location may be within the peripheral vasculature. Melanocortins have been observed to improve cardiovascular function and survival time in experimental hemorrhagic shock in various species. Though ACTH-(1–24) is the most potent melanocortin in this model, α-MSH and [Nle^4,D-Phe^7]α-MSH and ACTH-(4–10) are quite effective as well. As ACTH-(4–10) is a rather weak agonist of all melanocortin receptors, it is difficult to determine via which of the melanocortin receptors the melanocortins bring about this effect. Research into the nature of the receptors involved in the various cardiovascular effects of the melanocortins would greatly benefit from the availability of selective melanocortin receptor antagonists.

Keywords: Melanocortin; α-MSH (α-melanocyte-stimulating hormone); ACTH-(1–24) (adrenocorticotropic hormone-(1–24); γ_2-MSH (γ_2-melanocyte-stimulating hormone); Blood pressure; Heart rate; Cerebrocortical blood flow; Circulatory shock; Melancortin receptor; Melanocortin receptor subtype; FMRFamide receptor; (Rat)

1. The scope of this review

The melanocortins form a family of peptides derived from the common precursor pro-opiomelanocortin (POMC) that have the melanocyte-stimulating hormone (MSH) core sequence, His–Phe–Arg–Trp, in common (for an extensive review, see Eberle, 1988). Endogenous peptides belonging to this family are adrenocorticotropin (ACTH), α-MSH (NAc-ACTH-(1–13)-NH_2), β-MSH and, probably, Lys-γ_2-MSH. These peptides are generated by sequential processing from the POMC precursor (Eberle, 1988;

* Corresponding author. Tel.: +31-30-2538843; Fax: +31-30-2539032; E-mail: d.h.g.versteeg@med.uu.nl

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see Fig. 1). In addition to hormonal effects and effects on behavior, temperature regulation and natriuresis (see Eberle, 1988), members of the melanocortin family have been reported to have a variety of cardiovascular effects in various species. The pressor and tachycardiac effects of the γ-MSHs, peptides derived from the N-terminal part of POMC (Nakanishi et al., 1979), have been studied in detail and will be reviewed here.

In 1992, the first reports were published concerning the cloning of receptors for ACTH/MSH-like peptides, the α-MSH or melanocortin MC_1 receptor (Chhajlani and Wikberg, 1992; Mountjoy et al., 1992) and the ACTH or melanocortin MC_2 receptor (Mountjoy et al., 1992). Since then, three more subtypes of this family of receptors have been discovered (for reviews, see Low et al., 1994; Tatro, 1996; Adan and Gispen, 1997). Results of binding studies and of work on functional characterisation suggest that it is the melanocortin MC_3 receptor for which the γ-MSHs are selective (Gantz et al., 1993a; Roselli-Rehfuss et al., 1993; Adan et al., 1994a; also see reviews by Low et al., 1994 and Adan and Gispen, 1997).

The sites and mechanisms of action of the melanocortins are reviewed with respect to their effects on cardiovascular functions, with emphasis on the effects of the γ-MSHs. Present knowledge concerning agonist selectivity and localisation in the brain and peripheral tissues of the five melanocortin receptor subtypes cloned so far is the starting point. To this end we first describe the cardiovascular effects of the melanocortins, then briefly summarise the characteristics of the five known melanocortin receptor subtypes. The question as to whether or not the melanocortin MC_3 receptor subtype is indeed the receptor involved in the cardiovascular actions of the γ-MSHs is addressed next. We review the evidence concerning the structural features that determine the cardiovascular selectivity of the γ-MSHs. Available data concerning the site(s) and mechanism(s) of the γ-MSHs effects on blood pressure and heart rate are summarised. We conclude with a suggestion that melanocortin MC_4 receptors in the medulla oblongata are involved in cardiovascular regulation, possibly together with melanocortin MC_2 receptors in the peripheral vasculature. The fact that the structure–activity relationships for the cardiovascular effects are different from those for in vitro melanocortin receptor binding and second messenger production led us to postulate the existence in the brain of at least one, possibly two, still to be discovered receptors which are part of the central circuitry involved in cardiovascular control by melanocortins. These receptors need not be melanocortin receptors. We review evidence suggesting that these still unknown receptors could be related to other receptors for which the Arg–Phe sequence has affinity, such as the neuropeptide FF receptor (Payza et al., 1993) and the recently discovered FMRFamide receptor (Lingueglia et al., 1995).

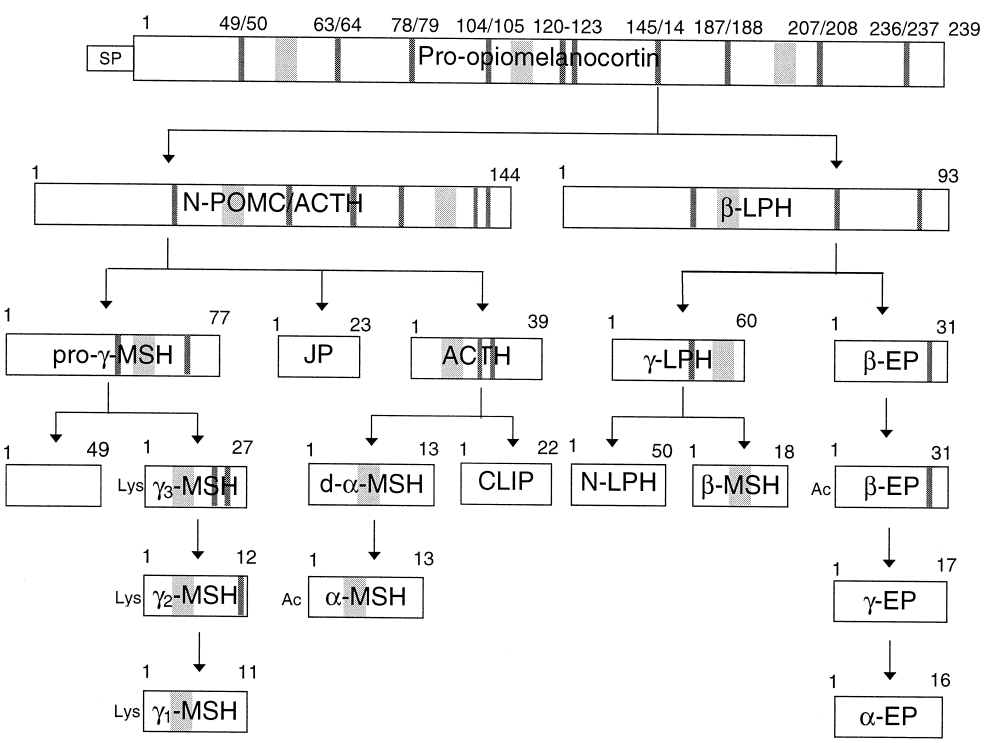

Fig. 1. POMC processing in the melanotrophs of the pars intermedia of the bovine pituitary. Modified from Eberle (1988). (Shaded) = MSH core sequence; (darkly shaded) = dibasic cleavage site (Arg–Lys, Lys–Lys, Lys–Arg or Arg–Arg).

2. Effects of γ-MSH / ACTH analogs

2.1. Pressor and tachycardiac effects of γ-MSH / ACTH-(4–10) analogs

The γ-MSHs have been found to have marked pressor and tachycardiac effects in the rat. The crucial observation in this respect was made by Gruber and co-workers (for a review, see Gruber and Callahan, 1989). Gruber and co-workers observed that γ_2-MSH and its des-Gly^{12} analog, γ_1-MSH, were 10 times more potent than ACTH-(4–10) to induce a dose-dependent, short-lasting increase in blood pressure and heart rate following their intravenous administration to conscious rats (Gruber et al., 1984; Klein et al., 1985; Gruber et al., 1985; Gruber and Eskridge, 1986; for amino acid sequences, see Table 1). Peptides with a short C-terminal extension containing the Arg–Phe sequence seem to have enhanced cardiovascular activity (Gruber and Callahan, 1989). Related peptides with a longer C-terminal extension with (γ_3-MSH) or without the Arg–Phe sequence (α-MSH) are devoid of these cardiovascular effects (Klein et al., 1985; for a review, see Gruber and Callahan, 1989; for amino acid sequences, see Table 1). These findings have been confirmed and extended added to by Sun et al. (1991, 1992), De Wildt et al. (1993, 1994, 1995), Versteeg et al. (1993), Li et al. (1996) and Van Bergen et al. (1995, 1996, 1997a,b, 1998). Fig. 2 (De Wildt et al., 1993), shows the pressor and tachycardiac effects of γ_2-MSH and ACTH-(4–10) in conscious, freely moving rats. These effects are accompanied by an increase in pulse amplitude (De Wildt et al., 1993). α-MSH as well as [MetO_2 ^{4},D-Lys^8,Phe^9]ACTH-(4–9) (Org 2766), a stable ACTH-(4–9) analog which has a variety of effects on behavior (for reviews see De Wied and Jolles, 1982; Van Nispen and Greven, 1986), are devoid of effects on blood pressure and heart rate (Fig. 2).

2.2. Depressor effects of γ-MSH / ACTH-(4–10) analogs

Gruber and Callahan (1989) have suggested that the cardiovascular effects of ACTH-(4–10)/γ-MSH-like peptides should be studied in conscious rats, as anesthetics suppress the sympathetic outflow, thus blunting the cardiovascular effects of the peptides. We, therefore, used rats under light urethane anesthesia, known to maintain reflexes and sufficient sympathetic tone, and found that γ_2-MSH caused haemodynamic responses similar to those observed in conscious rats (De Wildt et al., 1993). Rats under deep pentobarbital-induced anesthesia, however, showed a marked depressor effect of γ_2-MSH combined with a slight bradycardia (De Wildt et al., 1993). It is highly likely that under these circumstances anesthesia inhibits the pressor and chronotropic effects of the peptide by suppressing sympathetic tone. Melanocortins of the γ-MSH family appear to have two different effects on blood pressure and heart rate, each with a different underlying mechanism. The pressor and tachycardiac effects are predominant in conscious rats and in rats under mild urethane anesthesia, whereas the depressor effect appears only when the sympathetic drive to the periphery is sufficiently suppressed (De Wildt et al., 1993, 1994).

2.3. Depressor and bradycardiac effects following microinjection of melanocortins into medullary cardiovascular regulation centers

Depressor and bradycardiac effects followed the microinjection of γ_2-MSH locally into the commissural part of the nucleus tractus solitarii of rats under urethane anesthesia (De Wildt et al., 1994). Whereas the pressor and tachycardiac effects of γ_2-MSH following i.v. administration have a rapid onset and are short-lasting, with a maximal effect 25 s following administration (Sun et al., 1992; Versteeg et al., 1993; De Wildt et al., 1993, 1994), these same effects following microinjection of the peptide into the nucleus tractus solitarii develop gradually over a period of minutes and last for 10–15 min (De Wildt et al., 1994). Interestingly, time–response studies showed that the acute pressor response to a high dose of γ_2-MSH given i.v. to conscious rats is followed by a small but relatively long-lasting decrease in mean arterial pressure (Versteeg et al., 1993). While Li et al. (1996) reported that microinjection of α-MSH into the medullary dorsal–vagal complex results in a depressor and bradycardiac response as well, they found γ_2-MSH less effective in this respect.

2.4. Effects of γ-MSH / ACTH-(4–10) analogs on carotid and cerebral blood flow

We have shown that members of the γ-MSH family cause an increase in macro- and microcirculatory cerebral blood flow as well (De Wildt et al., 1995; Van Bergen et al., 1996). The effects of γ_2-MSH on cerebrocortical blood flow were twice as high after intracarotid than after i.v. infusion, which suggests a central site and mechanism for the effect (De Wildt et al., 1995). These effects of the γ-MSHs also are acute and short-lasting (De Wildt et al., 1995; Herz et al., 1996). Org 2766 was not effective in this respect (Herz et al., 1998).

2.5. Cardiovascular effects of ACTH-(1–24)

The cardiovascular effects of ACTH-(1–24) (for amino acid sequence see Table 1) are clearly different from those of the γ-MSHs. Following acute i.v. administration of ACTH-(1–24) a depressor effect is observed in rats (Nakamura et al., 1976; Van Bergen et al., 1996, 1997a) and rabbits (Szabo et al., 1989; Ludbrook and Ventura, 1995). This effect is not dependent on the presence of the adrenals, since it still occurs in bilaterally adrenalectomised rats (Nakamura et al., 1976; Van Bergen et al., 1997a). In rats and dogs subjected to hemorrhagic shock,

Table 1
Amino acid sequences of various melanocortins and of FMRFamide and neuropeptide FF

Peptide	Amino acid sequence																									
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
γ_1-MSH			**Tyr-**	Val-	**Met-**	Gly-	**His-**	**Phe-**	**Arg-**	**Trp-**	Asp-	**Arg-**	**Phe**														
γ_2-MSH			**Tyr-**	Val-	**Met-**	Gly-	**His-**	**Phe-**	**Arg-**	**Trp-**	Asp-	**Arg-**	**Phe-**	Gly													
Lys-γ_2-MSH		Lys-	**Tyr-**	Val-	**Met-**	Gly-	**His-**	**Phe-**	**Arg-**	**Trp-**	Asp-	**Arg-**	**Phe-**	Gly													
γ_3-MSH			**Tyr-**	Val-	**Met-**	Gly-	**His-**	**Phe-**	**Arg-**	**Trp-**	Asp-	**Arg-**	**Phe-**	Gly-	Arg-[a]	Arg-	Asn-	Gly-	Ser-	Ser-	Ser-	Gly-	Val-	Gly-	Ala-	Ala-	Gln
γ-MSH-(6–12)								**Phe-**	**Arg-**	**Trp-**	Asp-	**Arg-**	**Phe-**	Gly													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
α-MSH	Ac-	Ser-	**Tyr-**	Ser-	**Met-**	Glu-	**His-**	**Phe-**	**Arg-**	**Trp-**	Gly-	Lys-	Pro-	Val-NH_2													
NDP-MSH	Ac-	Ser-	**Tyr-**	Ser-	Nle-	Glu-	**His-**	D-Phe-	**Arg-**	**Trp-**	Gly-	Lys-	Pro-	Val-NH_2													
ACTH-(4–10)					**Met-**	Glu-	**His-**	**Phe-**	**Arg-**	**Trp-**	Gly																
ACTH-(1–24)		Ser-	**Tyr-**	Ser-	**Met-**	Glu-	**His-**	**Phe-**	**Arg-**	**Trp-**	Gly-	Lys-	Pro-	Val-	Gly-	Lys-	Lys-	Arg-	Arg-	Pro-	Val-	Lys-	Val-	Tyr-	Pro		
FMRFamide										Phe-	Met-	**Arg-**	**Phe**-NH_2													
Neuropeptide FF						Phe-	Leu-	Phe-	Gln-	Pro-	Gln-	**Arg-**	**Phe**-NH_2													

γ-MSHs and the α-MSH/ACTH-like peptides numbering is that for the amino acids in the sequence. Please note the difference in the numbering of the His–Phe–Arg–Trp MSH core sequence.
[a]Glycosilated.

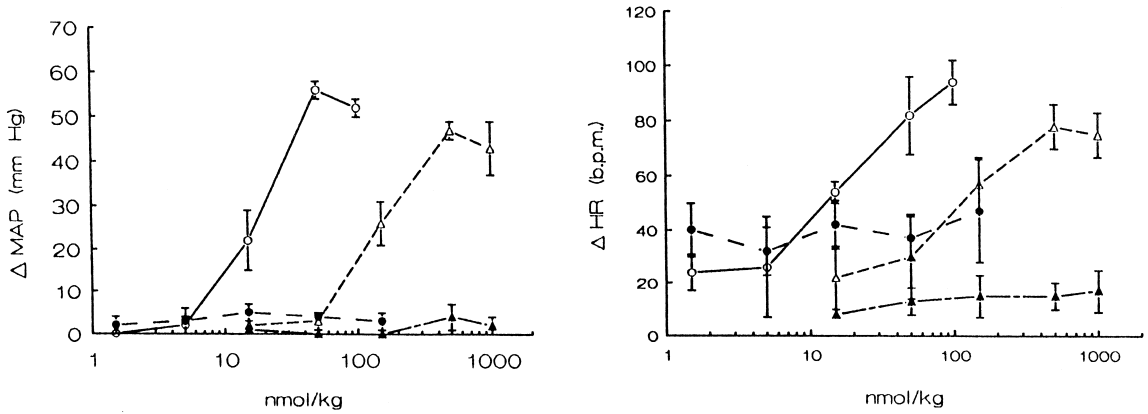

Fig. 2. Dose–response relationship for the effects of γ_2-MSH (○), ACTH-(4–10) (△), α-MSH (●) and Org 2766 (▲) on mean arterial pressure (MAP, A), heart rate (HR, B) after i.v. administration to conscious, freely moving rats. The data are expressed as absolute change from pre-administration values and as means ± S.E.M., $n = 6–8$. Reproduced from De Wildt et al. (1993).

ACTH-(1–24) given i.v. improves blood pressure, pulse amplitude and survival rate (Bertolini et al., 1986a,b). A similar effect has also been observed in humans (Bertolini et al., 1987; Noera et al., 1989; Pinelli et al., 1989). The structure–activity relationship for this effect differs from that for the effects on blood pressure and heart rate: smaller ACTH fragments, devoid of corticotropic activity, e.g., ACTH-(4–10), also increase the survival rate, but in this case, α-MSH also is as effective (Bertolini et al., 1986b; Coppi and Falcone, 1992). Finally, it has been reported that chronic administration of ACTH-(1–24) causes an increase in blood pressure in the rat (Haack et al., 1978; Freeman et al., 1980), sheep (Scoggins et al., 1984a,b), dog (McCaa et al., 1978) and man (Whitworth, 1992). This latter effect seems to be an indirect effect, caused by adrenal steroids (for a review, see Parkes et al., 1994).

3. Melanocortin receptor subtypes

Five subtypes of the melanocortin receptor family have been discovered; all belong to the superfamily of G-protein-coupled cell-surface receptors, have seven membrane-spanning domains and are coupled to adenylate cyclase. MSH/ACTH-like peptides selectively bind to and activate these receptors (for reviews see Low et al., 1994; Tatro, 1996; Adan and Gispen, 1997).

3.1. The melanocortin MC_1 receptor

This melanocortin receptor has been cloned in various species (Chhajlani and Wikberg, 1992; Mountjoy et al., 1992; Vanetti et al., 1994). It is expressed in cells of the melanocytic source (Chhajlani and Wikberg, 1992; Mountjoy et al., 1992; Chhajlani, 1996) and in the testis (Vanetti et al., 1994; Chhajlani, 1996). The effects of α-MSH on pigmentation via melanocytes are mediated by the melanocortin MC_1 receptor.

3.2. The melanocortin MC_2 receptor

This melanocortin receptor is the receptor for ACTH. The human, murine and bovine melanocortin MC_2 receptors have been cloned (Mountjoy et al., 1992; Raikhinstein et al., 1994; Kubo et al., 1995). This receptor is expressed in the adrenal cortex, especially in the zona fasciculata (site of glucocorticoid production) and the cortical zona glomerulosa (site of aldosterone synthesis). Some expression is also observed in the zona reticularis (Mountjoy et al., 1992). The melanocortin MC_2 receptor binds only ACTH and none of the other melanocortins (Schioth et al., 1996a).

3.3. The melanocortin MC_3 receptor

The human, rat and mouse melanocortin MC_3 receptors have been cloned (Gantz et al., 1993a; Roselli-Rehfuss et al., 1993; Desarnaud et al., 1994). This receptor is expressed in various brain parts. Melanocortin MC_3 receptor mRNA is expressed in the cortex, thalamus, septum, hippocampus, olfactory cortex, amygdala, periaqueductal gray, ventral tegmental area, interfascicular nuclei, central linear raphe nucleus and the hypothalamus (Gantz et al., 1993a; Roselli-Rehfuss et al., 1993; Kistler-Heer et al., 1998). mRNA is also expressed in the anteroventral periventricular nucleus and posterior hypothalamic area, regions which have been implicated in the neural control of cardiovascular and thermoregulatory functions (Roselli-Rehfuss et al., 1993; Low et al., 1994). However, no melanocortin MC_3 receptor mRNA expression was found in the nucleus tractus solitarii, another important site for cardiovascular control (Roselli-Rehfuss et al., 1993; Low et al., 1994). This receptor is also expressed in various peripheral tissues, such as stomach, gastro-intestinal tract and placenta, but not in the heart, kidney, lung, liver, testis, and melanoma cells (Gantz et al., 1993a; Roselli-Rehfuss et al., 1993; Desarnaud et al., 1994). Recently, the melanocortin MC_3 receptor was found in human heart (Chhajlani, 1996).

3.4. The melanocortin MC_4 receptor

The human and rat melanocortin MC_4 receptors have been cloned (Gantz et al., 1993b; Mountjoy et al., 1994). Melanocortin MC_4 receptor mRNA is present in regions throughout the brain, particularly in the isocortex, olfactory cortex, hippocampus amygdala, septal region, corpus striatum, nucleus accumbens, hypothalamus, nucleus tractus solitarii and the dorsal horn of the spinal cord (Mountjoy et al., 1994; Kistler-Heer et al., 1998). It is not expressed in the cerebellum and in the pancreas, heart, ovaries, testis, spleen, kidney, lung, stomach, gastro-intestinal tract and adrenal gland (Gantz et al., 1993b; Mountjoy et al., 1994).

3.5. The melanocortin MC_5 receptor

The human, rat, mouse and sheep melanocortin MC_5 receptors have been cloned (Chhajlani et al., 1993; Barrett et al., 1994; Gantz et al., 1994; Griffon et al., 1994; Labbe et al., 1994; Fathi et al., 1995). This receptor is expressed in the brain, in cortex, cerebellum, hippocampus, hypothalamus, substantia nigra and in the pituitary, and in various peripheral tissues, such as skeletal muscle, adrenal gland, stomach and lacrimal gland (Chen et al., 1997; Van der Kraan et al., 1998), but not in placenta, heart, kidney or gastrointestinal tract (Griffon et al., 1994; Labbe et al., 1994).

4. Which of the melanocortin receptor subtypes are involved in the cardiovascular effects of the melanocortins?

The melanocortin MC_3 receptor is the only melanocortin receptor subtype for which γ_2-MSH has affinity at nanomolar concentrations (Gantz et al., 1993a; Roselli-Rehfuss et al., 1993; Adan et al., 1994a; Desarnaud et al., 1994). γ_2-MSH has a much lower affinity for the melanocortin MC_5 receptor (Gantz et al., 1994; Labbe et al., 1994; Barrett et al., 1994; Schioth et al., 1996b), and the peptide has virtually no affinity for the other three subtypes (for reviews, see Low et al., 1994; Adan and Gispen, 1997). This makes the melanocortin MC_3 receptor an obvious leading candidate for mediation of the cardiovascular effects of the γ-MSHs. Two approaches have been followed to address this point: (1) comparison of the in vivo cardiovascular effects of various potent melanocortin receptor agonists, such as α-MSH, [Nle^2,D-Phe^7]α-MSH (NDP-MSH) and ACTH-(1–24), with those of γ_2-MSH, and (2) comparison of structure–activity relationships as exist for cardiovascular parameters in vivo, using a variety of γ-MSH-fragments (see above) with the same relationships for melanocortin receptor binding and activation in vitro.

4.1. Effects of α-MSH, NDP-MSH and ACTH-(1–24): comparison with those of the γ-MSHs

γ_2-MSH (= Tyr–Val–Met–Gly–His–Phe–Arg–Trp–Asp–Arg–Phe–Gly) (Klein et al., 1985; Gruber et al., 1985; Gruber and Eskridge, 1986; Sun et al., 1991, 1992; De Wildt et al., 1993, 1995; Versteeg et al., 1993; Van Bergen et al., 1995, 1996, 1997a; Li et al., 1996), its des-Gly^{12} analog, γ_1-MSH (Gruber et al., 1985; Sun et al., 1992) and Lys-γ_2-MSH (Van Bergen et al., 1995, 1996) are the melanocortins which increase blood pressure and heart rate most potently (see Section 2.1). ACTH-(4–10) (= Met–Glu–His–Phe–Arg–Trp–Gly), an ACTH/MSH fragment with micromolar affinity for all melanocortin receptor subtypes except the melanocortin MC_2 receptor (Chhajlani and Wikberg, 1992; Gantz et al., 1993a,b; Roselli-Rehfuss et al., 1993; Adan et al., 1994a; Desarnaud et al., 1994; Griffon et al., 1994; Labbe et al., 1994; Mountjoy et al., 1994; Schioth et al., 1995), is 5–10 times less potent than these three γ-MSHs to induce a pressor and a tachycardiac response (Klein et al., 1985; Gruber et al., 1985; Gruber and Eskridge, 1986; De Wildt et al., 1993; Versteeg et al., 1993; Van Bergen et al., 1995, 1996; see Fig. 2). The stable ACTH-(4–9) analog, Org 2766, which has no appreciable affinity for the melanocortin MC_1, MC_3, MC_4 and MC_5 receptor subtypes (Tatro and Entwistle, 1994; Adan et al., 1994a; Schioth et al., 1995), is ineffective (De Wildt et al., 1993; see Fig. 2).

α-MSH (NAc-ACTH-(1–13)-NH_2), which has affinity for all melanocortin receptor subtypes except the melanocortin MC_2 receptor, has no effect on blood pressure and heart rate in conscious rats (Gruber and Callahan, 1989; De Wildt et al., 1993; Li et al., 1996; see Fig. 2). The α-MSH analog, NDP-MSH (Sawyer et al., 1982), is the most potent agonist for the melanocortin MC_1, MC_3, MC_4 and MC_5 receptor (Gantz et al., 1993a; Chhajlani et al., 1993; Mountjoy et al., 1994). This peptide has, however, no effect on blood pressure and heart rate in conscious (Van Bergen et al., 1997a) and in urethane-anesthetized rats (Van Bergen et al., 1996; see Fig. 3). The lack of effect of α-MSH and NDP-MSH, both agonists with affinity for four of the five cloned melanocortin receptors, among them the melanocortin MC_3 receptor, on blood pressure and heart rate is a first argument against the notion that the pressor and tachycardiac effects of the γ-MSHs are either due to activation of the melanocortin MC_3 receptor, or are exerted via one of the other four known melanocortin receptor subtypes. This notion is supported by the findings of Li et al. (1996) that the pressor and tachycardiac effects of i.v. γ_2-MSH are not inhibited by the prior intracarotid administration of the melanocortin MC_3 receptor antagonist SHU9005 (Ac-[Nle^4,D-Phe(pI)^7,Lys^{10}]α-MSH-(1–13)-NH_2; Hruby et al., 1995).

Whereas melanocortin MC_4 receptors are expressed in the nucleus tractus solitarii (Mountjoy et al., 1994), melanocortin MC_3 receptors are not (Roselli-Rehfuss et

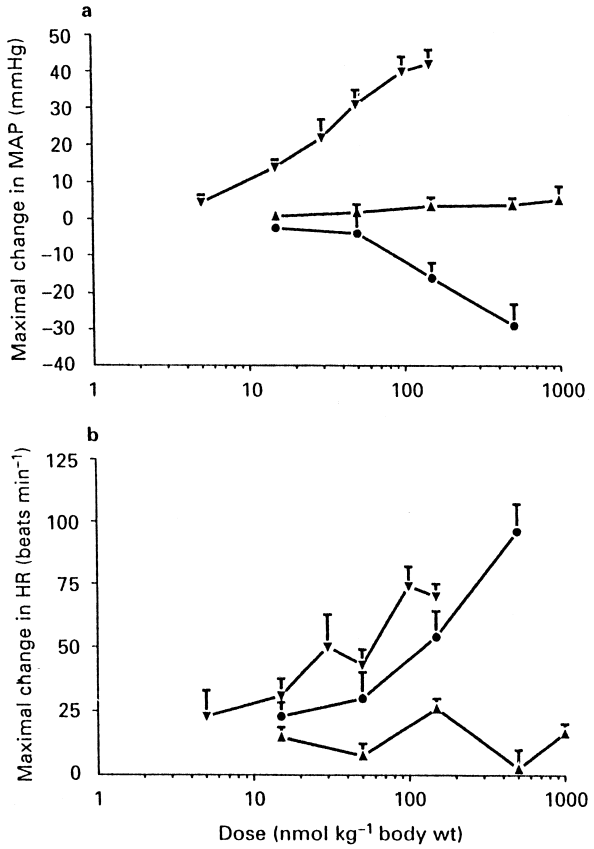

Fig. 3. Dose–response curves for the effects of NDP-MSH (▲, $n = 6$), ACTH-(1–24) (●, $n = 6$) and γ_2-MSH (▼, $n = 8$) following i.v. administration to conscious rats. Data are expressed as maximal change in MAP (A) and heart rate (B), and represent means ± S.E.M. Reproduced with permission from Van Bergen et al. (1997a).

al., 1993; Low et al., 1994). This means that the depressor and bradycardiac effects observed following microinjection of γ_2-MSH into the commissural part of the nucleus tractus solitarii (see Section 2.3), a brain region relatively rich in (Lys-)γ_2-MSH-like immunoreactivity (Fodor et al., 1996), are not mediated by melanocortin receptors of the MC_3 receptor subtype (De Wildt et al., 1994). Li et al. (1996) have reported that microinjection of α-MSH into the medullary dorsal–vagal complex results in a depressor and bradycardiac response, and that γ_2-MSH was less effective in this respect. Microinjection into this region of SHU9119 (Ac-Nle^4-c[Asp^5,D-Nal(2)^7,Lys^{10}]α-MSH-(4–10)-NH_2) did not alter blood pressure and heart rate (Li et al., 1996). SHU9119 is a cyclic lactam analog of α-MSH which combines potent antagonistic properties at the melanocortin MC_4 receptor and less potent antagonistic at the melanocortin MC_3 receptor with full agonistic properties at melanocortin MC_1 and MC_5 receptors (Hruby et al., 1995). Prior microinjection of SH9119, however, inhibited the depressor and bradycardiac effects of α-MSH microinjection in this region (Li et al., 1996). Li et al. (1996) interpret these results as indicating that it is via activation of melanocortin MC_4 receptors that α-MSH microinjected in this medullary region causes its depressor and bradycardiac effects.

As described in Section 2.4, γ_2-MSH (De Wildt et al., 1995; Van Bergen et al., 1996; Herz et al., 1998) and various γ-MSH analogs, including Lys-γ_2-MSH (Van Bergen et al., 1996), cause an increase in extra- and intracranial blood flow and in microcirculatory cerebrocortical blood flow in the urethane-anesthetized rat. Neither NDP-MSH nor ACTH-(1–24) had an effect on these variables, however (Van Bergen et al., 1996). These observations lead one to conclude that, also for the effects γ-MSH/ACTH-peptides on cerebral hemodynamics, it is not likely that one of the known melanocortin receptor is involved.

As described in Section 2, ACTH-(1–24) shows selectivity for the melanocortin MC_2 receptor subtype, though it activates the other subtypes as well. The melanocortin MC_2 receptor is, however, activated by ACTH only (Mountjoy et al., 1992; Raikhinstein et al., 1994; Kubo et al., 1995; Schioth et al., 1996a). Administration of ACTH-(1–24) to rabbits causes a dose-dependent decrease in blood pressure with a concomitant increase in heart rate (Szabo et al., 1989; Ludbrook and Ventura, 1995). This depressor effect seems to be due to a direct systemic vasodilatation, since the peptide was equally effective in the pithed rabbit with and without electrical stimulation (Szabo et al., 1987, 1989). ACTH-(1–24) also causes a depressor response in the conscious rat (Nakamura et al., 1976; Van Bergen et al., 1997a; see Fig. 3) in combination with a reflectory increase in heart rate (Van Bergen et al., 1997a; see Fig. 3). A similar decrease in blood pressure was induced by the peptide in the urethane-anesthetized rat, but without an effect on heart rate (Van Bergen et al., 1996). An action of ACTH-(1–24) on the adrenals appears not to be involved in the depressor effect, since bilateral adrenalectomy did not influence this depressor effect (Nakamura et al., 1976; Van Bergen et al., 1997a). Interestingly, administration of ACTH-(1–24) to the pithed rat, a model which excludes central activity, results in a depressor effect of the same magnitude (Van Bergen et al., 1998), whereas in the pithed rat γ_2-MSH has no effect on blood pressure and heart rate (Sun et al., 1992; Van Bergen et al., 1998). Hence, the depressor effect caused by ACTH-(1–24) has to be the result of an interaction of this peptide with a melanocortin receptor within the peripheral circulation. Since this effect is only observed after i.v. ACTH-(1–24) administration, it is likely that this receptor is of the MC_2 subtype.

4.2. Structure–activity studies with γ-MSH / ACTH-(4–10) fragments

4.2.1. Effects on blood pressure and heart rate

A variety of γ-MSH/ACTH-(4–10) fragments have been tested for their effects on blood pressure and heart rate in freely-moving, conscious rats as well as in ure-

thane-anesthetised rats (Van Bergen et al., 1995, 1996). The results of these structure–activity analyses yielded important clues as to which structural characteristics determine the cardiovascular selectivity of the γ-MSHs.

Whereas γ_1-MSH (= des-Gly^{12}-γ_2-MSH) is as effective as γ_2-MSH to induce a pressor and tachycardiac effect (Klein et al., 1985; Gruber et al., 1985), further C-terminal shortening results in fragments, γ-MSH-(1–10) and γ-MSH-(1–8), devoid of effects on blood pressure and heart rate (Van Bergen et al., 1995, 1996). Stepwise shortening of the γ_2-MSH chain from the N-terminal side yields fragments that lose potency progressively from γ-MSH-(2–12) down to γ-MSH-(5–12), with respect to the effects on blood pressure and heart rate, while their intrinsic activity is not different from that of γ_2-MSH (Van Bergen et al., 1995, 1996). Surprisingly, γ-MSH-(6–12) appeared to be more potent than γ_2-MSH (Van Bergen et al., 1995, 1996) (Fig. 4). The shortest fragment which displays pressor and tachycardiac effects is the MSH core, His–Phe–Arg–Trp (= γ-MSH-(5–8) = ACTH-(6–9)) (Van Bergen et al., 1995, 1996). Together, these findings indicate that the message essential for pressor and tachycardiac effects resides in the γ-MSH-(5–9)/ACTH-(6–9) sequence. C-terminal extension of this sequence with the sequence, Asp–Arg–Phe, establishes full intrinsic activity. The amino acid sequence N-terminal to the MSH core sequence appear to be essential for potency (Van Bergen et al., 1995, 1996).

4.2.2. Effects on extra- and intracranial blood flow

γ_2-MSH causes an strong increase (see Section 2.4.) in internal and total carotid blood flow and in cerebrocortical blood flow following both intracarotid and intravenous administration to urethane-anesthetised rats (De Wildt et al., 1995), an effect which is absent following administration of either NDP-MSH or ACTH-(1–24) (Van Bergen et al., 1996). As was found for the effects on blood pressure, it appeared that the presence of the C-terminal extension of the MSH core sequence, His–Phe–Arg–Trp, with the sequence Asp–Arg–Phe is essential (Van Bergen et al., 1996) for intrinsic activity regarding the effects on extra- and intracranial blood flow and central blood flow.

N-terminal shortening of the γ_2-MSH sequence had similar consequences for the effects on total carotid blood flow (Van Bergen et al., 1996) and for the effects on blood pressure and heart rate. This, however, does not apply for the effect on internal carotid flow (macrocirculatory intracerebral blood flow) and cerebral blood flow (microcirculatory cerebrocortical blood flow) (Van Bergen et al., 1996). γ-MSH-(4–12), γ-MSH-(5–12) and γ-MSH-(6–12) were as effective as γ_2-MSH and Lys-γ_2-MSH to increase the values of these parameters (Van Bergen et al., 1996; see Fig. 4). There, thus, appears to be a difference in the rank order and potency for the peripheral effects (blood pressure, heart rate, total carotid flow) on the one hand and for the central hemodynamic effects (internal carotid flow and cerebral blood flow) on the other. This suggests that different mechanisms and/or receptor subtypes are involved.

4.2.3. In vitro effects on receptor binding and cAMP production

Comparison of the results for the cardiovascular effects as described above (see Sections 4.2.1 and 4.2.2) with the structure–activity relationships found for in vitro receptor binding and cAMP production, provides further evidence suggesting that the cardiovascular effects of the γ-MSHs following their i.v. administration are not mediated by the melanocortin MC_3 receptor. γ-MSH-(6–12) tested in vitro on the melanocortin MC_3, MC_4, and MC_5 receptor did not activate any of these three melanocortin receptors (Adan et al., manuscript in preparation, Table 2), whereas Lys-γ_2-MSH does activate the melanocortin MC_3 receptor (Adan et al., 1994a). Other γ-MSH/ACTH-(4–10) fragments also had different abilities to activate the various melanocortin receptors in vitro and in vivo (Table 2; Van Bergen et al., 1995, 1996; Adan et al., manuscript in preparation). These results, especially those for γ-MSH-(6–12), are further support for the postulate that the cardiovascular effects of γ_2-MSH are most likely to be mediated by an as yet to be discovered receptor. γ-MSH-fragment (6–12) may well be the key tool for the identification of the receptor mediating the cardiovascular effects of γ_2-MSH and its analogs.

4.3. Is a receptor related to the FMRFamide receptor involved?

Mues et al. (1982) reported that peripheral administration of Phe–Met–Arg–Phe–NH_2 (FMRFamide, see Table 1), a cardioactive peptide first isolated from extracts of the ganglia of the clam *Macrocallista nimbosa* by Price and Greenberg (1977), results in increases in blood pressure and heart rate in the rat, with a time-course identical to that seen after administration of γ_1-MSH. The authors provided evidence that the effects of FMRFamide are dependent on the carboxyterminal Arg–Phe configuration (Mues et al., 1982). Dockray et al. (1983) subsequently identified a related pentapeptide, Leu–Pro–Leu–Arg–Phe–NH_2 (LPLRFamide), in the chicken brain, using antibodies against FMRFamide. It appeared that the C-terminal Arg–Phe–NH_2 sequence of both these peptides is identical to the C-terminal dipeptide of γ_1-MSH. Another member of this family of peptides with this C-terminal Arg–Phe–NH_2 sequence was isolated by Yang et al. (1985) from bovine brain, using antisera against FMRFamide. This peptide, Phe–Leu–Phe–Gln–Pro–Gln–Arg–Phe–NH_2 (FLFQPQRFamide, also termed F8Famide, morphine-modulating peptide and neuropeptide FF, see Table 1), is widely distributed in the mammalian brain (Kivipelto et al., 1989; Kivipelto and Panula, 1991; Allard et al., 1992; Kivipelto

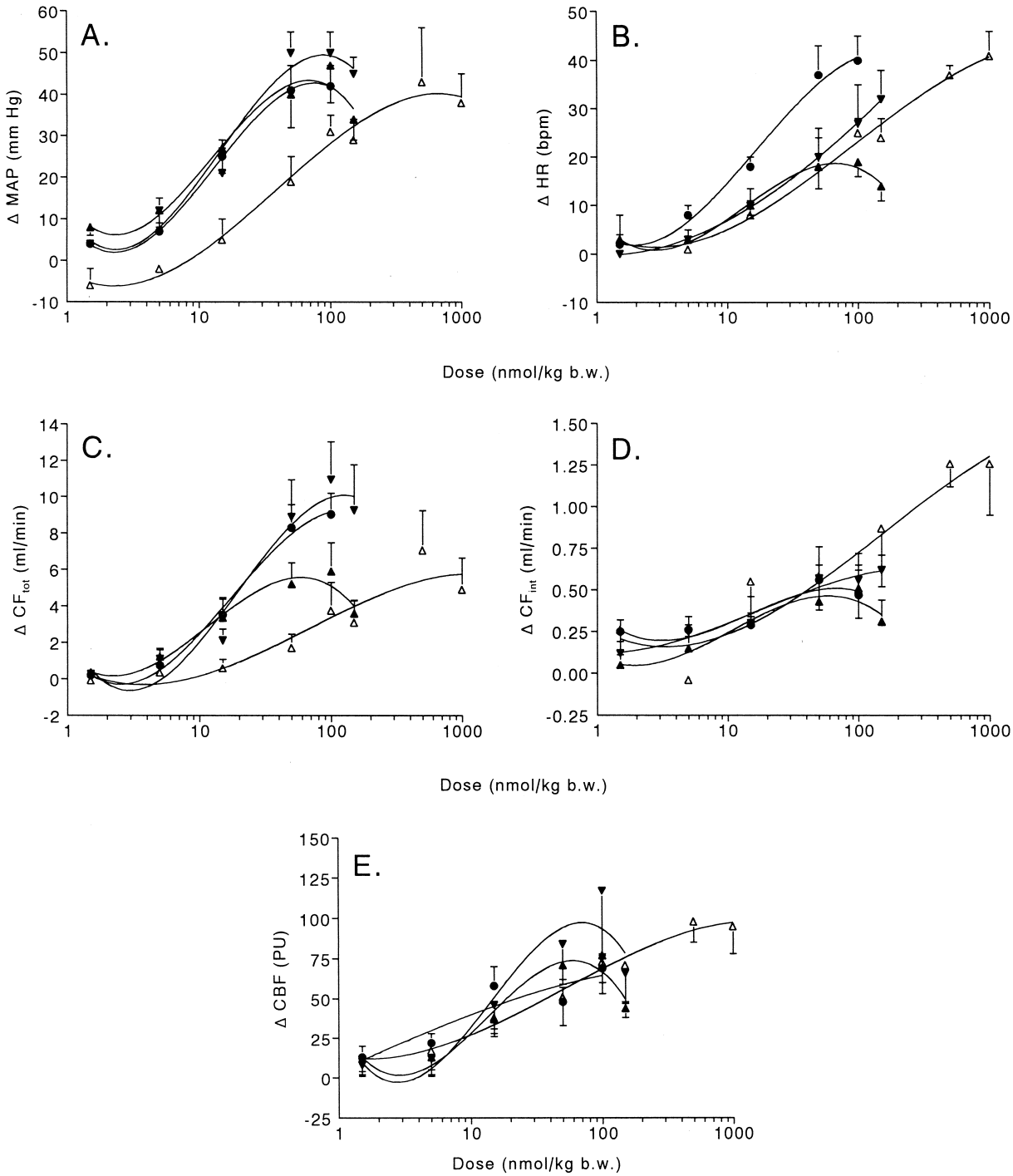

Fig. 4. Dose–response relationship for the effects of γ_2-MSH (●) and for γ-MSH analogs with N-terminal chain shortening (γ-MSH-(4–12) (▲); γ-MSH-(5–12) (△); γ-MSH-(6–12) (▼)) on mean arterial pressure (MAP, A), heart rate (HR, B), total carotid blood flow (CF_{tot}, C), internal carotid blood flow (CF_{int}, D) and cortical cerebral blood flow (CBF, E) after intracarotid administration to urethane-anesthetised rats. The data are expressed as absolute change from pre-administration values and as means ± S.E.M., $n = 6–10$. Reproduced from Van Bergen et al. (1996).

et al., 1992; for a recent review, see Roumy and Zajac, 1998). Both LPLRFamide (Barnard and Dockray, 1984) and neuropeptide FF (Roth et al., 1987; Allard et al., 1995) cause an increase in blood pressure and heart rate following their systemic administration. Thiemermann et al. (1991) studied the effects in rats of a series of FMRFamide congeners and found that of the dipeptide, Arg–Phe, was the most potent pressor peptide in this series. The authors concluded that, since these peptides were not effective in pithed rats, they were increasing blood pressure and heart rate by central activation of the sympathetic nervous system (Thiemermann et al., 1991).

Table 2
Semi-quantitative comparison of the in vivo and in vitro activity of α-MSH, Lys-γ_2-MSH and fragments of the latter peptide with N-terminal chain shortening

Peptide	In vivo activity	In vitro activity		
		MC_3	MC_4	MC_5
α-MSH	−	+ + +	+ + +	+ + +
Lys-γ_2-MSH	+ +	+ + +	+	+
γ-MSH-(2–12)	+ +	+ + +	+	+ / −
γ-MSH-(5–12)	+	−	−	−
γ-MSH-(6–12)	+ + +	−	−	−
γ-MSH-(5–8)	+ / −	−	−	−

The in vivo effect corresponds to the effects of the melanocortins on blood pressure in conscious and urethane-anesthetised rats (see Sections 4.2.1 and 4.2.2).
In vitro activity was tested on human and rat/mouse melanocortin MC_3, MC_4 and MC_5 receptors expressed in 293 HEK cells.
The assay makes use of an *lac*Z gene which is expressed under the control of a cAMP-regulated promotor in a pCRElacZ construct to detect changes in intracellular cAMP concentrations as a result of receptor activation (Adan et al., manuscript in preparation).
The code for the activity is: + + + = very strong; + + = strong; + = moderate; + / − = weak; − = no activity.

Thus, there are striking similarities in chemical structure and effects of peptides belonging to the FMRFamide/neuropeptide FF series and the γ-MSH family of peptides. FMRFamide binds to the neuropeptide FF receptor (Payza et al., 1993), a receptor which is widely distributed in brain regions involved in autonomic information processing (Dupouy and Zajac, 1996). There are reports of the characterisation and isolation of a FMRFamide receptor of the squid *Loligo pealei* (Chin et al., 1994) and of cloning of an FMRFamide-gated Na^+ channel (Lingueglia et al., 1995). It is tempting to speculate that receptors of the FMRFamide/neuropeptide FF receptor family rather than melanocortin receptors are involved in the cardiovascular effects that follow systemic administration of γ-MSHs.

5. The cardiovascular effects of the melanocortins: sites of action

There follows an attempt to reconcile what is known about the structure–activity relationships for the cardiovascular effects of the melanocortins and the resulting speculations about the receptors involved in these effects with the evidence as to the mode and site of action of these peptides to induce changes in cardiovascular functions (see Fig. 5). It will have become clear that it is highly likely that more than one melanocortin receptor subtype is involved in the cardiovascular effects of the melanocortins. In addition, it seems likely that non-melanocortin receptors are involved in mediating the effects of the γ-MSHs as well. A possible candidate in this respect is, as just mentioned, a receptor related to the FMRFamide or neuropeptide FF receptor.

Results of pharmacological experiments support the notion that both pressor and chronotropic activities of the γ-MSHs depend on the maintenance of sympathetic drive to the vascular system and the heart. Pre-treatment with the α_1-adrenoceptor antagonist, prazosin, results in a marked reduction of the pressor effect of γ_2-MSH (Callahan et al., 1984; Van Bergen et al., 1997b), while pre-treatment with the β_1-adrenoceptor antagonist metoprolol shifts the dose–response curve for the tachycardiac effect of the peptide to the right (Van Bergen et al., 1997b). Ganglionic blockade with chlorisondamine reduced by 80% the pressor response to γ_2-MSH, indicating that the effects of the peptide are mediated through the preganglionic sympathetic drive (Callahan et al., 1988c). Such results led Gruber and Callahan to postulate that the cardiovascular effects of i.v. administered γ-MSHs result from activation of central rather than of peripheral mechanisms (for a review, see Gruber and Callahan, 1989). The likelihood that a primary site for the pressor/tachycardiac action of the γ-MSHs is within the central nervous system is supported by the absence of an effect of γ_2-MSH on blood pressure and heart rate in pithed rats (Sun et al., 1992; Van Bergen et al., 1998). It is thought that the primary site of action of the peptides is located in the anteroventral third ventricle (AV3V) region, a forebrain regions situated outside the blood-brain barrier, therefore, to which circulating peptides have access (Weindl, 1973; Brody et al., 1984). The information is then relayed from the AV3V region to

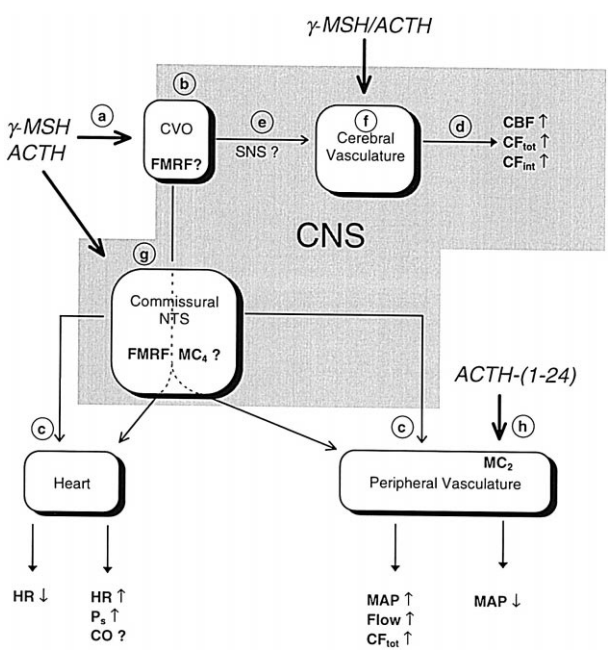

Fig. 5. Proposed sites of action of melanocortins with respect to their cardiovascular effects. See text for explanation. CBF = cerebral blood flow; CF_{int} = internal carotid flow; CF_{tot} = total carotid flow; CNS = central nervous system; CO = cardiac output; CVO = circumventricular organs; FMRF = FMRFamide receptor; HR = heart rate; MAP = mean arterial pressure; MC = melanocortin receptor; NTS = nucleus tractus solitarii; P_s = systolic blood pressure; SNS = sympathetic nervous system.

the medullary vasomotor nuclei by a descending pressor pathway which is supposedly vasopressinergic (Ciriello and Calaresu, 1980; Ferguson and Renaud, 1984; Ferguson et al., 1984; Knupfer et al., 1984) with the consequence that the preganglionic sympathetic drive to the periphery is increased (for details and literature, see Gruber and Callahan, 1989). Various findings support this notion. The cardiovascular effect of a given dose of γ_2-MSH is significantly more pronounced after intracarotid than after i.v. administration (Callahan et al., 1988b; De Wildt et al., 1995; Li et al., 1996; Van Bergen et al., 1997a). Lesions in the AV3V region produce a shift to the right of the dose–response curve for the pressor response to i.v. administered γ_2-MSH (Callahan et al., 1988a). Thus, following i.v. or i.car. administration of γ_2-MSH or its active analogs, the peptides are transported to the AV3V region (Fig. 5a). There, they activate a receptor which could be either a still to be discovered melanocortin receptor subtype or a FMRFamide/neuropeptide FF-like receptor (Fig. 5b).

It is very likely that, with the involvement of brain vasopressin receptors of the V_{1B} subtype (Gruber and Eskridge, 1986; Van Bergen et al., 1997b), activation of this forebrain receptor is followed by enhancement of the sympathetic outflow to the peripheral target organs (Fig. 5c; Callahan et al., 1985; Sun et al., 1992; De Wildt et al., 1993), resulting in a sympathetic nervous system-mediated increase in heart rate and blood pressure (Callahan et al., 1984; Van Bergen et al., 1998). The pressor response induced in conscious rats by γ_2-MSH and its cardiovascular active fragments is the consequence of an increase in peripheral vascular resistance as measured in the mesenteric and hindlimb beds, but not in the renal bed (Callahan et al., 1985).

Activation of a melanocortin receptor or of another type of receptor within the AV3V might lead to active vasodilation in the cerebral macro- and microvasculature (Fig. 5d; De Wildt et al., 1995; Van Bergen et al., 1996), since the flow-enhancing effect of γ_2-MSH and its active analogs appears to be rather independent of the perfusion pressure-elevating effects of these peptides. It can be speculated that stimulation of the sympathetic nervous system (Fig. 5e) activates cerebrovascular β_2-adrenoceptors, an activation which ultimately resulting in cerebral vasodilation. Another possibility is that the vasodilatation is due to a direct interaction with receptors located in the cerebral vasculature (Fig. 5f). In this respect, it is of interest that Xia and Wikberg (1997) recently found intense γ_2-MSH-like immunoreactivity in cerebral blood vessels. Further, a melanocortin receptor is expressed by mouse brain microvascular endothelial cells in culture (De Angelis et al., 1995). The subtype of this receptor is not yet known, though the authors suggest that it is an α-MSH receptor.

There is evidence for the nucleus tractus solitarii as a target for γ_2-MSH (Fig. 5g; De Wildt et al., 1994). Stimulation of receptors in this brain area, especially in the commissural part of the nucleus tractus solitarii, is able to induce a depressor and bradycardiac response (De Wildt et al., 1994). Whether this cardiovascular action is the consequence of reduced activity of the sympathetic nervous system or of activation of a vagal component needs further study. While melanocortin MC_4 receptors occur in the nucleus tractus solitarii region of the brain, melanocortin MC_3 receptors do not (Roselli-Rehfuss et al., 1993; Mountjoy et al., 1994; Low et al., 1994). γ_2-MSH has low affinity for the melanocortin MC_4 receptor (Gantz et al., 1993b; Adan et al., 1994a; Mountjoy et al., 1994; Schioth et al., 1995). It is still unknown whether, in the nucleus tractus solitarii, the peptide acts via the melanocortin MC_4 receptor or a yet to be discovered receptor subtype.

Since NDP-MSH produces a depressor effect only after its intracarotid administration (Van Bergen et al., 1996) and since α-MSH can cause a hypotensive effect when injected in lower brain structures i.e., the dorsal–vagal complex (Li et al., 1996), it is conceivable that melanocortin MC_4 receptors in the brainstem are involved in the depressor action by interference with the vagal outflow. The overall effect after i.v. administration of γ-MSH/ACTH fragments whether via stimulation of melanocortin receptors within higher brain structures (the circumventricular organs), leading to a higher sympathetic nervous system outflow, or involving within lower brain areas (the nucleus tractus solitarii), probably affecting vagal outflow, appears to depend on the state of arousal of the rat. For example, there is either a depressor response (during deep anesthesia; or full activation of the nucleus tractus solitarii when injected locally), a pressor response (during the conscious state, when forebrain centers override the nucleus tractus solitarii-mediated action), or biphasic responses (higher incidence after intracarotid administration than after i.v. administration to urethane-anesthetized rats; the commissural part of the nucleus tractus solitarii is more activated and thus overrides forebrain centers).

The depressor effects of ACTH-(1–24) are shared by none of the other melanocortins after i.v. administration (see above). With NDP-MSH a depressor effect is only observed after high doses given intracarotid, which suggests that this effect is the result of activation of a central melanocortin receptor (see above). Since ACTH-(1–24) is fully effective to induce a depressor effect in the pithed rat model (Van Bergen et al., submitted), it is likely that a melanocortin MC_2 receptor in the peripheral vascular system is responsible for the acute blood pressure lowering effects of ACTH (Fig. 5h). However, nothing is known concerning the occurrence of melanocortin MC_2 in the peripheral vasculature.

As was summarised in Section 1, ACTH and related peptides have been found to improve cardiovascular function and survival time in rats subjected to experimental hemorrhagic shock (Bertolini et al., 1986a,b). Though ACTH-(1–24) is the most potent melanocortin, α-MSH

and NDP-MSH and ACTH-(4–10) are also quite effective (Bertolini et al., 1986b). It is, therefore, difficult to determine via which of the melanocortin receptors the melanocortins exert their beneficial effect in this model.

6. Concluding remarks

It will have become clear that the melanocortins have multiple effects on cardiovascular function and that various melanocortin receptor subtypes are involved in these effects. These receptors include the melanocortin MC_2 receptor, presumably in the peripheral vasculature, and the MC_4 receptor, in the medulla oblongata. It is postulated that there are one or more still to be discovered receptors, most likely located in the AV3V region and the nucleus tractus solitarii region of the brain, and possibly, in the cerebral vasculature so as to make the cardiovascular effects of the γ-MSHs explainable. A non-melanocortin receptor belonging to a group of receptors for FMR-Famide- and/or neuropeptide-related peptides may be involved in at least some of the cardiovascular effects of the γ-MSHs. Characterisation of these receptors is of crucial importance to future research in this field. A key peptide for identifying the receptor involved in the cardiovascular effects of the γ-MSHs might be γ-MSH-(6–12), which is the most potent γ-MSH fragment to induce a pressor and bradycardiac response (Van Bergen et al., 1995, 1996). It is essential that selective agonists and, even more important, antagonists for the known receptors, as well as for those yet to be discovered be synthetised (see Adan et al., 1994b; Hruby et al., 1995).

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