γ-MSH, sodium metabolism, and salt-sensitive hypertension

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Humphreys, Michael H. γ-MSH, sodium metabolism, and salt-sensitive hypertension. Am J Physiol Regul Integr Comp Physiol 286: R417–R430, 2004; 10.1152/ajpregu.00365.2003.—α-, β-, and γ-melanocyte stimulating hormones (MSHs) are melanotropin peptides that are derived from the ACTH/β-endorphin proopiomelanocortin (POMC). They have been highly conserved through evolutionary development, although their functions in mammals have remained obscure. The identification in the last decade of a family of five membrane-spanning melanocortin receptors (MC-Rs), for which the melanotropins are the natural ligands, has permitted the characterization of a number of important actions of these peptides, although the physiological function(s) of γ-MSH have remained elusive. Much evidence indicates that γ-MSH stimulates sympathetic outflow and raises blood pressure through a central mechanism. However, this review focuses on newer cardiovascular and renal actions of the peptide, acting in most cases through the MC3-R. In rodents, a high-sodium diet (HSD) increases the pituitary abundance of POMC mRNA and of γ-MSH content and results in a doubling of plasma γ-MSH concentration. The peptide is natuuretic and acts through renal MC3-Rs, which are also upregulated by the HSD. Thus the system appears designed to participate in the integrated response to dietary sodium excess. Genetic or pharmacologic induction of γ-MSH deficiency results in marked salt-sensitive hypertension that is corrected by the administration of the peptide, probably through a central site of action. Deletion of the MC3-R also produces salt-sensitive hypertension, which, however, is not corrected by infusion of the hormone. These observations in aggregate suggest the operation of a hormonal system important in blood pressure control and in the regulation of sodium excretion. The relationship of these two actions to each other and the significance of this system in humans are important questions for future research.

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Invited Review

POMC METABOLISM AND PROCESSING

POMC is best known as the prohormone of ACTH and β-endorphin. It is heavily expressed in corticotrophs of the anterior lobe of the pituitary and in melanotrophs of the intermediate lobe. However, it is also expressed in other parts of the central nervous system (CNS), notably the arcuate nucleus of the hypothalamus and the nucleus of the solitary tract and in extracranial tissues, including the skin, testis, thyroid, placenta, pancreas, gastrointestinal tract, kidney, and liver (21, 111). It is the product of a single gene composed of three exons separated by two introns, a structure similar among all mammalian species. Current understanding holds that circulating POMC-derived peptides are secreted from the pituitary, whereas peptides in extrapituitary tissues function in an autocrine or paracrine manner.

Processing of POMC into its component peptides is driven by two prohormone convertases, PC1 and PC2. These are subtilisin-like endopeptidases that cleave polypeptides at sites of dibasic amino acid pairs (Lys-Lys, Lys-Arg, Arg-Arg, Arg-Lys), and all the POMC-derived peptides are flanked by such pairs. PC1 is expressed predominately in anterior lobe corticotrophs and to only a low extent in intermediate lobe (20, 132); it cleaves POMC into the larger peptides ACTH, β-lipotropin, and the large NH2-terminal fragment. PC2 is more prominent in melanotrophs of the intermediate lobe but is also expressed at low levels in anterior lobe corticotrophs; its action gives rise to the smaller peptides β-endorphin and α-, β-, and γ-MSH (132). Other enzymes are involved in COOH-terminal amidation and NH2-terminal acetylation of some of these peptides to complete processing.
Regulation of POMC synthesis and processing occurs through markedly different mechanisms in anterior and intermediate lobes. In anterior lobe corticotrophs, the major stimulus for POMC synthesis is CRF from the hypothalamus (4, 111). This peptide induces a rapid increase in POMC gene transcription and its mRNA. Glucocorticoids exert a negative feedback control on POMC gene expression by inhibiting POMC gene expression directly and also reduce the expression of PC1 and PC2 (20). In the intact animal, synthesis of POMC is the resultant of CRF-mediated stimulation balanced by glucocorticoid inhibition. A number of other regulatory factors including vasopressin, catecholamines, serotonin, and angiotensin II have also been shown to regulate POMC expression (4, 111).

Regulation in the neurointermediate lobe (NIL) is mediated primarily by the neurotransmitter dopamine acting through the dopamine D2 receptor. In NIL, this receptor is negatively coupled to adenylate cyclase; activation of the receptor inhibits POMC gene expression and secretion of derived peptides, whereas antagonism of the receptor upregulates POMC expression (4, 111). Abundance of PC1 and PC2 mRNA and protein move in parallel with POMC mRNA in response to dopaminergic stimulation or antagonism (20, 94). These processing enzymes act in a coordinate manner to mediate specific endoproteolytic cleavages of POMC at dibasic amino acid pairs in a strict temporal order, with PC1 acting initially and PC2 later to generate the smaller peptides secreted from the NIL (132, 133). PC2 requires the coexpression of the neuroendocrine protein 7B2 for normal activation (6) (see below).

**THE MELANOCORTINS AND THEIR RECEPTORS**

\(\alpha\)- and \(\beta\)-MSH were identified in the 1950s because of their action to induce pigment dispersion in skin melanocytes of amphibia and reptiles. In 1979, Nakagishi and colleagues (83) deduced the amino acid sequence of the whole POMC molecule from its complementary DNA. They identified in the NH2-terminal fragment a region bearing homology with \(\alpha\)- and \(\beta\)-MSH in a core heptapeptide. Because this region was separated by dibasic amino acid cleavage sites, they predicted that the intervening peptide would be a secretory product of POMC and termed it \(\gamma\)-MSH because of this homology to \(\alpha\)- and \(\beta\)-MSH. They surmised that it too would possess the pigmen
tory capabilities of its two relatives. This has proven not to be the case, but much work has revealed that \(\gamma\)-MSH possesses important cardiovascular actions not shared by its cousins. Three species of \(\gamma\)-MSH have been recognized (Fig. 1). \(\gamma_1\)-MSH contains 11 amino acids and is identical to \(\gamma_2\)-MSH without the COOH-terminal glycine and with COOH-terminal amidation. \(\gamma_2\)-MSH is a 25-amino acid peptide formed by cleavage at the next dibasic amino acid cleavage site. The functional significance of these different forms of \(\gamma\)-MSH is not completely clear but has bearing on their cardiovascular actions, as will be described shortly.

As discussed earlier, \(\alpha\)- and \(\gamma\)-MSH are processed chiefly in the intermediate lobe and secreted into the circulation. The pigmen
tory function of \(\alpha\)-MSH in mammals is minor, yet the highly conserved nature of POMC fueled a search for other functions of these peptides, and it has become clear that they are involved in a wide variety of important actions. This work was facilitated by the cloning of a family of five receptors with which the peptides interact (Table 1). These receptors are members of the superfam
yily of seven-transmembrane-spanning receptors and share considerable amino acid homology among themselves. They are positively coupled to adenylate cyclase, and their activation results in an increase in cellular cAMP (80, 107). However, other signaling pathways have been identified, including activation of inositol trisphosphate with mobilization of intracellular calcium (59, 79), an influx of extracellular calcium (58), and the MAP kinase pathway (33). The MC1-R is the receptor mediating the classical effects of \(\alpha\)-MSH on melanin dispersion; it is expressed by cutaneous melanocytes but is also found on monocytes and other immunomodulatory cells and has been proposed to mediate the anti-inflammatory actions of \(\alpha\)-MSH (41, 107). Loss-of-function mutations of MC1-R in humans are relatively common and are associated with red hair color (110). The MC2-R is expressed in the zona

![Fig. 1. A: overview of the proopiomelanocortin (POMC) molecule showing the dibasic amino acid sites of processing into the hormones derived from it. B: amino acid sequences of melanocyte stimulating hormone (MSH) peptides. \(\alpha\)-, \(\beta\)-, and \(\gamma\)-MSH share a core heptapeptide sequence. Three species of \(\gamma\)-MSH have been recognized as described in the text. [Borrowed with permission of the American Heart Association (54a).]
fasciculata and zona reticularis of the adrenal cortex and is responsible for the stimulation of glucocorticoid synthesis by ACTH. It binds ACTH with high affinity and is responsible for the stimulation of glucocorticoid synthesis by ACTH. Mutations in MC2-R result in autosomal recessive familial isolated glucocorticoid deficiency (19, 116, 125).

The MC3-R is expressed in brain, chiefly in hypothalamus, cortex, and thalamus, as well as in peripheral tissues including gut and placenta (22, 42, 104). In the hypothalamus, it is found in the arcuate nucleus, an important site of extrapituitary POMC synthesis and processing. It has also been detected in human and rat kidney (15, 85), which is relevant to the natriuretic actions of the melanocortins to be discussed below. Of the five melanocortin receptors cloned to date, γ-MSH has affinity in the nanomolar range only for MC3-R, leading to the conclusion that it is the natural ligand for this receptor. Deletion of the MC3-R gene by homologous recombination in the mouse leads to a condition of idiopathic hyperaldosteronism (see below). Aldosterone synthesis takes place in the zonal glomerulosa and is transiently regulated by ACTH. Mutations in MC2-R result in autosomal recessive familial isolated glucocorticoid deficiency (19, 116, 125).

The MC4-R is expressed in brain, chiefly in hypothalamus, cortex, and thalamus, as well as in peripheral tissues including gut and placenta (22, 42, 104). In the hypothalamus, it is found in the arcuate nucleus, an important site of extrapituitary POMC synthesis and processing. It has also been detected in human and rat kidney (15, 85), which is relevant to the natriuretic actions of the melanocortins to be discussed below. Of the five melanocortin receptors cloned to date, γ-MSH has affinity in the nanomolar range only for MC3-R, leading to the conclusion that it is the natural ligand for this receptor. Deletion of the MC3-R gene by homologous recombination in the mouse leads to a condition of idiopathic hyperaldosteronism (see below). Aldosterone synthesis takes place in the zonal glomerulosa and is transiently regulated by ACTH. Mutations in MC2-R result in autosomal recessive familial isolated glucocorticoid deficiency (19, 116, 125).

The MC4-R is heavily expressed in almost all brain regions and in the spinal cord (41, 107). It has highest affinity for α- and β-MSH but rather low affinity for γ-MSH, and the current viewpoint is that α-MSH is its natural ligand. Targeted disruption of the MC4-R gene results in a phenotype of hyperphagia, obesity, and insulin resistance (56). The MC5-R is expressed at low levels in numerous tissues including skeletal muscle, brain, adrenal gland, kidney, lung, testis, and uterus (41, 107). It is also detected in exocrine glands like the Harderian, preputial, sebaceous, and lacrimal glands and is found as well in prostate and pancreas. Targeted deletion of this receptor gene results in a widespread impairment of exocrine gland function manifested by defects in water expulsion and thermoregulation (13).

Table 1 also lists the relative affinities of the major POMC-derived melanotropin peptides for the five receptors. These data are summaries of results of in vitro binding experiments and measurements of CAMP generation in cellular systems and may not reflect activity in vivo. As will be pointed out later in this review, γ-MSH, acting through MC3-R, selectively corrects the salt-sensitive hypertension observed in γ-MSH-deficient rodents; equimolar infusions of α-MSH are without effect, even though the two peptides are reported to have similar affinities for MC3-R in vitro.

### Table 1. Characteristics of the melanocortin receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligands</th>
<th>Tissue Distribution</th>
<th>Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC1-R</td>
<td>α-MSH &gt; β-MSH &gt; γ-MSH &gt; ACTH</td>
<td>Melanocytes, macrophages</td>
<td>Skin pigmentation; immune modulation</td>
</tr>
<tr>
<td>MC2-R</td>
<td>ACTH</td>
<td>Adrenal cortex</td>
<td>Steroidogenesis</td>
</tr>
<tr>
<td>MC3-R</td>
<td>γ1-MSH = γ2-MSH = α-MSH = β-MSH &gt; γ-MSH</td>
<td>Hypothalamus, thalamus, other brain regions; kidney, gut, placenta, heart</td>
<td>Sodium metabolism, blood pressure regulation, energy homeostasis</td>
</tr>
<tr>
<td>MC4-R</td>
<td>α-MSH = β-MSH &gt; ACTH &gt; γ-MSH</td>
<td>Brain, spinal cord</td>
<td>Energy metabolism, food intake</td>
</tr>
<tr>
<td>MC5-R</td>
<td>α-MSH = ACTH &gt; β-MSH &gt; γ-MSH</td>
<td>Adrenal cortex, brain, lung, spleen, exocrine glands</td>
<td>Sebaceous gland function</td>
</tr>
</tbody>
</table>

MSH, melanocortin stimulating hormone; -R, receptor.

CARDIOVASCULAR ACTIONS OF γ-MSH

α-MSH and ACTH-(1–24) have been shown to exert resuscitative effects in models of hemorrhagic and septic shock (reviewed in Refs. 41, 107, 127). Some of these actions occur through a central site of action, whereas others appear to result from inhibition of production of cytokines like TNF-α. These anti-inflammatory actions inhibit acute renal and hepatic injury in rodent models (16, 17, 18); γ-MSH and structurally related peptides are not effective (127). On the other hand, γ-MSH-like peptides have been recognized to possess a variety of cardiovascular actions not shared by α- and β-MSH. Injections of γ-MSH intravenously or into a carotid artery result in a transient pressor and cardioaccelerator response that is due to central activation of sympathetic outflow. This may involve a vasopressinergic pathway, because it is blunted in Brattleboro rats lacking vasopressin (45), although not all observations are consistent with this (119). It likely also involves a neural circuit in the anteroverentral region of the third ventricle, because lesions of this region reduced the pressor response to intravenous γ-MSH (11). This part of the hypothalamus lies outside the blood-brain barrier and so can respond to intravenously delivered peptide. γ-MSH administered directly into the cerebroventricular system (icv) also raises blood pressure but over a different time course of 10 – 15 min as opposed to a few seconds (115). In general, α-MSH has no effect on blood pressure or heart rate when given intravenously (23, 101, 121). However, it elevates blood pressure and heart rate when given intracerebroventricularly (29, 47, 72). This effect results from stimulation of sympathetic outflow and is mediated via MC4-Rs (74, 75). In contrast, microinjection of α-MSH into the drososoreal complex of the medulla or the nucleus of the solitary tract lowers blood pressure and heart rate (65, 95), also likely mediated via MC4-R (65, 95). This action may help to explain the observation that acute intracerebroventricular administration of α-MSH raises blood pressure, whereas chronic administration lowers it (47). γ-MSH itself produced bradycardia and lowered blood pressure when injected directly into the nucleus of the solitary tract in contrast to its effects when given intravenously or intracerebroventricularly (24, 65). These observations indicate that the circuitry mediating the various cardiovascular actions of these peptides is complex; whereas the usual effect of γ-MSH peptides is to elevate blood pressure and heart rate, a vasodepressor effect can be manifest when they are injected directly into specific brain regions or when sympathetic outflow is depressed as under certain conditions of anesthesia (23, 44).
Structure-function studies have identified some features of MSH peptides that account for their cardiovascular activities. The sequence Arg-Phe at positions 10 and 11 of γ1- and γ2-MSH is necessary for the hypertensive effect of these peptides when administered intravenously, because analogs like α-MSH that lack them have no activity (Fig. 1). They must be at or near the COOH terminus, because γ2-MSH possesses this sequence but also has a long COOH-terminal extension and is inactive relative to the shorter forms when injected intravenously; however, it was equipotent to γ2-MSH in raising mean arterial pressure (MAP) when given into the cerebral ventricle (101), raising the possibility that its greater size in some way restricts its access to active sites in the central nervous system. Asp at position 9 confers selectivity for MC3-R relative to MC4-R (91). NH2-terminal shortening produces peptides that maintain cardiovascular activity, with γ-MSH (6-12) being if anything more potent than γ2-MSH (89, 120, 121, 122). These observations, and others, raise important questions about the melanocortin receptor(s) mediating these activities. As discussed above, the cardiovascular actions of α-MSH appear to be mediated by MC4-R. γ-MSH has affinity in the concentration range at which it circulates only for MC3-R, and it would be reasonable to consider this receptor to mediate the pressor and tachycardic actions of this group of peptides. However, Li and colleagues (65) found that the hypertensive effect of intravenous γ2-MSH was not blocked by intracarotid injection of the MC3-R and MC4-R antagonist SHU9005, and others observed that the ability of peptide fragments to bind to and activate the various receptors correlated very poorly with their effects on blood pressure and heart rate; indeed, γ-MSH (6-12) failed to activate in vitro the MC3-R, MC4-R, or MC5-R yet possessed potent activity in vivo (120, 121, 122). Such observations as these have led to the suspicion that the cardiovascular actions of γ-MSH and related peptides are mediated by an as yet undetected melanocortin receptor and/or a different class of receptor (65, 89, 123).

Speculation has centered on the FMRFamide-gated sodium channel as a candidate receptor. FMRFamide (Phe-Met-Arg-Phe-NH2) was originally isolated from ganglia of the clam Macrocristallana nimbosa (98), and a related peptide, neuropeptide FF (Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH2), later identified in bovine brain (Ref. 57; reviewed in Ref. 105). These and related peptides are pressor and cardioaccelerator when injected systemically through central activation of the sympathetic nervous system, with a time course identical to that of γ1-MSH (81, 115). Thiemann and associates (115) studied a number of related peptides and observed that the dipeptide Arg-Phe was the most potent pressor. Arg-Phe-NH2 is the NH2-terminal sequence of γ1-MSH; as discussed earlier, these two amino acids are necessary for the pressor actions of γ1-MSH and γ2-MSH. In 1995, Linguaglia and colleagues (71) reported the cloning of an amiloride-sensitive FMRFamide peptide-gated sodium channel from snail nervous tissue, for which FMRFamide and possibly related sympathoexcitatory peptides like γ1- and γ2-MSH could be the natural ligands. Subsequent work has suggested that activation of this system may be involved in the development of salt-sensitive hypertension (51, 90). These observations are relevant to the finding of salt-sensitive hypertension in γ-MSH-deficient rodents presented below. Although much more work needs to be done, the possibility exists that this amiloride-sensitive sodium channel could be the receptor activated by γ-MSH and related peptides to increase sympathetic outflow, raising blood pressure and heart rate. The availability of MC3-R-deficient mice (10) should allow definitive conclusions about the role of this receptor in the cardiovascular actions of MSH peptides; in a preliminary study, we observed that the cardiovascular responses to injections of γ-MSH are the same in wild-type and MC3-R knockout mice (86), a finding that argues against any role of MC3-R in these responses.

### γ-MSH and Natriuresis

Early studies by Orias and McCann (92), and subsequently by Hradec and Horky (49), demonstrated that α- and β-MSH were natriuretic when injected intraperitoneally into the rat and hamster. In 1984, Lymangrover and associates (73) showed that γ2-MSH also is natriuretic when injected as a bolus intravenously in doses from 0.64 to 64 pmol; doses higher than 64 pmol lost natriuretic activity. These groups of investigators concluded that the natriuretic effect of MSH peptides was likely a direct one on the kidney, although the systemic route of administration of the peptides did not allow a firm conclusion on this point. In 1987, Lin and colleagues (67) reported that γ2-MSH was natriuretic when infused directly into the renal artery at a rate that caused no change in sodium excretion from the contralateral kidney. This clearly indicated an intrarenal mechanism for the natriuresis; because no change in glomerular filtration rate occurred, the data were interpreted to indicate that the natriuresis resulted from inhibition of tubular sodium reabsorption (14, 67).

The mechanism of γ-MSH natriuresis has not been fully elucidated. Both mRNA and protein for MC3-R, MC4-R, and MC5-R have been identified in rat renal cortex and medulla (85), although their exact cellular localization has not been determined. Studies showed that the MC3-R and MC4-R antagonists SHU9005 and SHU9119 (50) infused intrarenally blocked the natriuresis induced by γ-MSH, supporting a receptor-mediated mechanism of action (14). Excretion rates of both cGMP (UcGMPV) and cAMP (UcAMPV) increased in proportion to the increase in sodium excretion after intravenous infusion of γ-MSH, and UcGMPV also rose after intrarenal γ-MSH infusion (14). Excretion rates of cyclic nucleotides are often used as indexes of hormonal action in the kidneys; as discussed earlier, melanocortin receptors are positively coupled to adenylate cyclase, and the increase in UcGMPV was therefore surprising. An increase in plasma atrial natriuretic peptide (ANP) concentration was observed after intravenous infusion of γ-MSH, so the possibility of an indirect effect of the peptide, acting through ANP to cause natriuresis, was raised (14). However, neutralization of secreted ANP by treatment with an anti-ANP antiserum did not alter the natriuresis caused by intravenous infusion of γ-MSH, and the authors concluded that the increase in plasma ANP resulting from γ-MSH infusion was not involved in the renal actions of this peptide (14). This same study also indicated that the renal nerves may be involved in the natriuretic effect of γ-MSH. Acute renal denervation blocked the natriuresis caused by intrarenal infusion of the peptide, whereas denervation did not alter the natriuresis resulting from intrarenal ANP infusion (14). The nature of this interaction between the renal nerves and γ-MSH-mediated natriuresis remains to be defined.

Invited Review

R420

γ-MSH and Circulatory Homeostasis
γ-MSH AND THE POSTNEPHRECTOMY NATRIURESIS

Interest in the possible role of γ-MSH as a physiological regulator of sodium metabolism arose from studies of the natriuresis that occurs after acute unilateral nephrectomy (AUN). The postnephrectomy natriuresis was first clearly described by Peters (97) and has been confirmed in numerous subsequent reports. The natriuresis from the remaining kidney occurs within minutes of AUN (26, 27, 100) and takes place without an increase in glomerular filtration rate (GFR), indicating that it results from a decrease in tubular sodium reabsorption (69, 70, 97, 103). Although a transient increase in MAP is observed after AUN (5, 26, 87; see below), there is no overall increase in blood pressure that could account for the natriuresis. Because there is no discernible change in the volume or composition of the blood after AUN, a neurohumoral mechanism was suggested to mediate the postnephrectomy natriuresis. Such a mechanism could be considered to represent the efferent limb of a reflex arc activated by AUN, the other components of which include afferent inputs and central integrative pathways.

Several components involved in this reflex natriuresis after AUN have been identified and are summarized in Fig. 2. The reflex is initiated by the abrupt rise in arterial pressure that occurs immediately after clamping of the renal artery (5, 26, 87; Fig. 2, #1). This in turn is the result of the hydrodynamic consequence of the removal of one organ vascular resistance circuit (the kidney) in parallel with the multiple other regional vascular resistance networks (the other kidney, brain, heart, skeletal muscle, splanchnic circulation, etc.) that contribute to total peripheral resistance. In a parallel circuit, removal of one component must inevitably lead to an increase in overall resistance. Because cardiac output initially is unchanged and maintains constant flow, the result of the increased resistance is an increase in arterial pressure sensed by high-pressure baroreceptors in the carotid sinus. This rise in blood pressure proved to be the signal initiating the reflex: prevention of this hydrodynamic consequence of AUN by opening a peripheral arteriovenous (AV) fistula at the time of AUN also prevented the postnephrectomy natriuresis (53), and both the hemodynamic changes and the excretory response resemble those seen on closure of a chronic AV fistula (52). Subsequent reflex effects reduce cardiac output and restore blood pressure back to control (52, 53, 54). Additional experiments established the importance of the carotid sinus baroreceptors (Fig. 2, #2): maneuvers that interfered with normal carotid sinus function or that prevented transmission of the increased pressure signal to the baroreceptors also prevented the postnephrectomy natriuresis (5). CNS involvement was more clearly established in the natriuretic response to AUN by experiments demonstrating that ventriculocisternal perfusion with pharmacologic agents blocked the natriuresis at doses that had no effect when given systemically (68; Fig. 2, #3). A role of the pituitary emerged from the finding that the postnephrectomy natriuresis did not occur in hypophysectomized animals (70; Fig. 2, #4). Finally, pituitary POMC became implicated when the plasma concentration of immunoreactive material from the midregion of the NH2-terminal fragment of POMC, termed NTF32–49, was observed to increase after AUN in a manner that was closely correlated with the increase in UNaV (70). Further evidence of a pituitary source for the secretion of this immunoreactivity came from studies in which a lesion of the arcuate nucleus of the hypothalamus was produced by neonatal administration of monosodium glutamate (69). The arcuate nucleus is an important neuroendocrine control station regulating pituitary function; rats treated in this manner exhibit a number of endocrine, metabolic, and behavioral disturbances attributed to altered pituitary function (84). Rats with this lesion failed to increase either UNaV or plasma NTF32–49 concentration after AUN. This lack of response was associated with a reduction in NTF-like material in the pituitary of these rats (69).

We speculated that γ-MSH could be the component of the NH2-terminal region of POMC that was associated with the postnephrectomy natriuresis, and we obtained evidence consistent with this possibility (67). AUN led to natriuresis that was accomplished by an increase in plasma γ-MSH immuno-
reactivity. This increase correlated with the increase in NTF22–40 (100) and with the increase in UnaV (67); as discussed above, the peptide itself was natriuretic when infused either intravenously or directly into one renal artery. Pretreatment with rabbit anti-γ-MSH antiserum blocked the natriuretic response to AUN (63). These observations therefore suggested that γ-MSH acted as a classic circulating hormone after AUN (Fig. 2, #5); its concentration in plasma (presumably reflecting increased secretion) rose after AUN but not after sham nephrectomy; it possessed natriuretic potency when infused intravenously or directly in one renal artery; and the presence of an antiserum, which likely bound and rendered inactive any freshly released peptide, prevented the postnephrectomy natriuresis. The characteristics of the antiserum used in these studies led to the conclusion that γ2-MSH, as opposed to γ1- or γ3-MSH, mediates the natriuretic response (55, 67, 100). Of note, no change in the plasma concentration of another POMC-derived peptide, ACTH, occurred after AUN, suggesting a selective effect of this maneuver on secretion of γ2-MSH. Moreover, no change in plasma ANP concentration was observed, indicating that activation of this potent natriuretic pathway was not responsible for the postnephrectomy natriuresis (100). Further support for mediation of the natriuresis after AUN by γ-MSH came from experiments in which the melanocortin receptor antagonists SHU9119 and SHU9005 were infused into the renal artery of the remaining kidney before carrying out AUN. These compounds have high potency and selectivity for the MC3-R and MC4-R while retaining full agonist activity at MC1-R and MC5-R (50). Intrarenal infusion of either compound prevented the postnephrectomy natriuresis despite equivalent elevations of plasma γ-MSH concentration (87), providing additional evidence for a receptor-mediated mechanism as being responsible for the natriuresis.

Mention was made earlier that renal denervation prevented the natriuretic response to infusion of γ-MSH. Similarly, renal denervation also prevented the postnephrectomy natriuresis (55, 103) but in a complex manner. Renal innervation appeared necessary for the nephrectomy to stimulate γ-MSH release into the circulation, a requirement that was attributed toafferent renal nerves (103; Fig. 2, #6).

γ-MSH AND SODIUM METABOLISM

The identification of a role of γ-MSH in the reflex natriuresis initiated by AUN suggested the possibility that this humoral system could be involved in a more general way in sodium metabolism. Indeed, the components of this reflex arc (baroreceptor inputs, hypothalamic integration, hypothalamic-pituitary axis) mediate other circumstances of body fluid homeostasis and circulatory control. Duchen (28) demonstrated an increase in the number of secretory granules in NIL but not anterior lobe (AL) of rats drinking hypertonic saline, and Howe and Thody (48) extended this observation by measuring an increase in NIL MSH content by bioassay in rats drinking 2% saline for 10–15 days. Elkabes and Loh (32) measured plasma α-MSH concentration and pituitary POMC mRNA and synthetic rate in mice drinking either tap water or 2% saline solution. They observed that plasma α-MSH concentration, reflecting secretion from the NIL, fell after 2 days of the high sodium intake but then returned to baseline from 4 to 12 days after starting the high intake. POMC mRNA abundance in the NIL was reduced after 2 days of saline ingestion and then returned to baseline; in contrast, it was elevated in the AL 2 days after introduction of the high sodium intake, returning to baseline at later time points (32). The authors interpreted their results to indicate that sodium ingestion exerts divergent effects on POMC metabolism in the different pituitary lobes and that salt loading may interact with other regulators of pituitary function such as stress to account for their observations (32).

These results thus provide an example where peptides from the N-terminal region of POMC may be processed and secreted differently from ACTH, in contrast to the conclusion drawn by Elkabes and Loh (32) that they reflect a nonspecific response driven by ACTH release resulting from the stress imposed by the HSD.

These findings suggested that the HSD has a preferential effect on pituitary POMC processing into γ-MSH and secretion of the peptide into the circulation. We quantitated POMC mRNA abundance in whole pituitaries of these rats and observed a progressive increase in message abundance after 1, 2, and 3 wk of ingesting the HSD (77); in situ hybridization studies indicated that the increase in mRNA abundance was largely confined to the NIL and was accompanied by a large increase in γ-MSH immunoreactivity in this lobe but not the AL (77; Fig. 3). A subsequent study confirmed the effect of the HSD to increase plasma γ-MSH concentration as well as the POMC signal in NIL and in addition demonstrated that the mRNAs of the processing enzymes PC1 and PC2, as well as PC2 protein abundance, were also increased in NIL but not AL after 3 wk of the HSD (12). In aggregate, these observations all point to a coordinated upregulation of the POMC-γ-MSH system in NIL in response to the HSD: the HSD initiates a signal that reaches the NIL to increase the mRNA abundance of POMC and the enzymes required for its processing into γ-MSH. Secretion of the peptide into the circulation increases its plasma concentration and results in natriuresis. This scheme suggests that the system is one component of the integrated response to states of sodium excess and thereby contributes to the maintenance of normal sodium balance.

To be natriuretic, the peptide must interact with receptors within the kidney. The absence of any change in GFR during γ-MSH natriuresis argued for a site of action on the renal epithelium where receptors must be located on the surface of renal tubular cells. Mentioned earlier were the reports of renal expression of MC3-R, the melanocortin receptor subtype with which γ-MSH interacts (15, 85). In the rat kidney, mRNA and protein for MC3-R, MC4-R, and MC5-R have been detected in both cortex and medulla in roughly equal abundance during ingestion of a LSD. During an HSD, both the mRNA and
protein abundance of MC3-R more than doubled in medulla but not cortex, whereas expression of the other receptors did not change (85). It thus seems that the HSD not only activates synthesis and secretion of γ-MSH from the NIL but also upregulates the expression of its receptors in the kidney, thereby amplifying the potency of its natriuretic action. This is in contrast to the ANP system, which is also activated by the HSD: plasma ANP concentration increases, but there is no change in the renal expression of the ANP receptor natriuretic peptide receptor (NPR)-A (37, 113). This dual action of the HSD to increase not only plasma γ-MSH concentration but also its receptor MC3-R in the kidney argues that this system is an important component of the integrated response to states of sodium surfeit.

γ-MSH AND SALT-SENSITIVE HYPERTENSION

The possibility of involvement of altered POMC metabolism in the pathogenesis of hypertension has been considered. Gaida and colleagues (40) found that β-endorphin immunoreactivity was increased in NIL of stroke-prone spontaneously hypertensive rats (SHR) and was not affected by antihypertensive treatment with clonidine, whereas Felder and Garland (35) observed that synthesis of POMC by hypertensive rat pituitary NIL was blunted compared with normotensive rats. Pharmacologic treatment of hypertensive rats with a variety of agents restored blood pressure to normal and also normalized POMC synthesis, leading the authors to conclude that the blunted synthesis while hypertensive was a consequence rather than a cause of the hypertension (35). These studies did not quantitate POMC mRNA, nor were the pituitary changes related to circulating concentrations of peptides derived from POMC. In contrast to these observations of altered POMC metabolism in NIL of hypertensive rats, Braas and associates (8) found reduced levels of POMC peptides and POMC mRNA in anterior lobes of hypertensive rats. In none of these studies was the temporal relationship of blood pressure changes to POMC metabolism examined or the consequence of alterations in sodium intake on these variables studied. Hao and Rabkin (46) reported still different results after measuring POMC mRNA in pituitaries of Dahl salt-resistant and salt-sensitive rats. They observed an increase in POMC mRNA abundance in resistant rats ingesting an HSD but not in salt-sensitive rats. They could not determine if this difference between salt resistant and salt sensitive was causally related to the hypertension of sensitive rats or a result of it, nor did they relate it to changes in circulating concentration of any POMC-derived peptides. Their study followed the earlier work of Rapp and Dahl (102), who showed decreased protein synthesis and α-MSH content in NIL in response to sodium loading in salt-sensitive compared with salt-resistant rats. We also observed in preliminary studies that Dahl salt-sensitive rats failed to increase pituitary POMC mRNA when ingesting the HSD and also did not increase plasma γ-MSH concentration (76). These last observations raise the possibility that impaired POMC metabolism may contribute to salt-sensitive hypertension.

The renal and cardiovascular effects of γ1- and γ2-MSH have also been studied in SHR and the normotensive control Wistar-Kyoto (WKY) rats (114). Both peptides caused dose-dependent increases in MAP and heart rate in conscious intact rats that were not observed in rats that had been pithed. These authors also presented data indicating that intravenous infusion of nonpressor doses of γ2-MSH led to equivalent natriuresis in untreated SHR and WKY, suggesting that the natriuretic component of the peptide’s actions was preserved in this form of genetically hypertensive rat. On the other hand, a preliminary study indicated that intrarenal infusion of γ2-MSH in untreated SHR did not exert a natriuretic action; treatment with the angiotensin-converting inhibitor captopril, added to the drinking water for 7–10 days, lowered MAP from 161 to 120 mmHg and restored natriuretic responsiveness to intrarenal infusion of γ2-MSH (131). The basis for these divergent observations on the renal action of γ-MSH in SHR has not been determined.
More recent studies in rodents with γ-MSH deficiency or resistance identify the importance of the γ-MSH system in sodium metabolism. Mention was made earlier of the importance of PC2 in the processing of POMC into its secretory product γ-MSH. Mice with targeted deletion of the PC2 gene were first reported in 1997 by Steiner’s group (38); these mice exhibit a modest impairment in growth and have lower blood glucose concentration than their wild-type littermates but are otherwise phenotypically normal. They demonstrate defective processing of a number of peptide hormones in addition to POMC and exhibit a defect in dopaminergic suppression of NIL ACTH (126). Adrenalectomy with steroid hormone replacement rescues the lethal phenotype of these mice, which also exhibit a defect in dopaminergic suppression of NIL ACTH secretion (62). Blood pressure was not reported in these studies, and it is not known if secretion of γ-MSH from the intermediate lobe was also impaired in 7B2 null mice. The basis for the phenotypic differences between PC2 knockout and 7B2 null mice is not clear, but these differences indicate that interruption of normal prohormone processing by PC2 can lead to pleiotropic effects.

The findings described above in PC2 knockout mice argue that γ-MSH deficiency results in salt-sensitive hypertension and document therefore the important role of this peptide in sodium metabolism. We sought to determine if γ-MSH resistance also resulted in a hypertensive phenotype by measuring MAP on the LSD vs. HSD in mice with targeted disruption of the MC3-R or MC4-R. Mc3r−/− mice exhibit a unique metabolic syndrome characterized by an increase in adipose tissue mass without obesity and with reduced energy expenditure (10), whereas Mc4r−/− mice are phenotypically obese with increased adipose tissue, hyperphagia, and insulin resistance (56). Mc3r−/− mice had a plasma γ-MSH concentration on the LSD that was more than double the value observed in wild-type controls, and the value increased even more in knockout mice ingesting the HSD. This suggested that these knockouts have a hormone-resistant state. MAP reflected the results in PC2−/− mice: it was somewhat higher in knockout mice on the LSD than in wild-type mice but became markedly hypertensive in Mc3r−/− mice that had been ingesting the HSD for 1 wk (88). In contrast to PC2−/− mice, infusion of γ-MSH had no effect on MAP in these Mc3r−/− mice, indicating that the restoration of MAP to normal by γ-MSH administration to hypertensive PC2−/− mice required integrity of this receptor. Mc4r−/− mice were normotensive on both the LSD and HSD (88).

In aggregate, these experiments paint a picture of a humoral system, the disruption of which, either by impairment of normal secretion or by blunting of its signaling through its...
receptor, results in salt-sensitive hypertension. Such a finding
demonstrates the importance of this system in normal sodium
metabolism. The mechanism(s) by which interruption of
\(\gamma\)-MSH signaling causes hypertension is not yet clear. Plasma
renin activity and plasma aldosterone concentration were
equivalently suppressed during ingestion of the HSD in
PC2\(-/-\) mice, arguing that these important determinants of
MAP were not involved. Intravenously infused \(\gamma\)-MSH increased
\(U_{Na}V\) in hypertensive PC2\(-/-\) mice as MAP was
falling to normal levels, but the rapidity of the blood pressure-
lowering effect of the peptide makes a reduction in plasma
volume from the natriuresis seem unlikely. Moreover, cere-
broventricular administration of the peptide led to even more
rapid correction of MAP in doses that had no effect given
intravenously, so that natriuresis could not be the mechanism
by which MAP was lowered. However, the natriuretic property of
the peptide could certainly interact with its blood pressure-
lowering action to participate in the overall regulation of the
circulation and body fluid volumes. The central site of action of
the peptide given to \(\gamma\)-MSH-deficient mice suggests that it may
possibly reduce central sympathetic outflow, an interesting
hypothesis in view of the literature cited earlier in this review
documenting the effect of \(\gamma\)-MSH, at much higher doses, to
stimulate sympathetic outflow. Obviously, much further work
will be required to characterize fully the mechanism(s) under-
lying salt-sensitive hypertension that occurs as a result of
impaired \(\gamma\)-MSH signaling.

The pleiotropic consequences of PC2 deficiency, as de-
scribed above, raise the concern that systems in addition to
\(\gamma\)-MSH could contribute to the hypertension that these animals
display. We consequently used a pharmacologic approach in an
effort to interfere with NIL processing of POMC without
perturbing other systems dependent on PC2. As discussed
earlier, the major pathway regulating NIL function involves
dopaminergic suppression. We treated male rats with the
dopamine agonist bromocriptine (5 mg/kg ip by daily injection)
or the dopamine receptor antagonist haloperidol (5 mg/kg ip
each day) for 1 wk while they were ingesting either the LSD or
the HSD and compared the results with those in vehicle-treated
rats. Vehicle-treated rats on the HSD showed an elevation in
plasma \(\gamma\)-MSH concentration and NIL \(\gamma\)-MSH content com-
pared with rats ingesting the LSD (78) as we had seen earlier
(77); MAP did not differ in the two groups. Haloperidol-treated
rats had elevated plasma and NIL \(\gamma\)-MSH levels on the LSD,
and these did not increase further on the HSD. MAP again was
no different in the two groups. Bromocriptine-treated
produced opposite results. Neither plasma \(\gamma\)-MSH concentra-
cion nor NIL \(\gamma\)-MSH content was elevated in bromocriptine-treated
rats on the HSD compared with LSD values, and, interestingly,
MAP was significantly higher in the HSD animals (132 ± 3 vs.
100 ± 3 mmHg in LSD rats, \(P < 0.001\)) (78). Thus dopami-
nergic stimulation with bromocriptine produced \(\gamma\)-MSH defi-
ciency during ingestion of the HSD, and this was accompanied
by the development of salt-sensitive hypertension. As was true
with \(\gamma\)-MSH-deficient PC2\(-/-\) mice, infusion of the peptide at
a physiologically relevant rate restored MAP to control
values very quickly (78). These results indicate that pharma-
cologic treatment known to interfere with POMC processing
results in \(\gamma\)-MSH deficiency and that this deficiency is associ-
ated with the occurrence of salt-sensitive hypertension.

Because other pathways requiring PC2 for prohormone pro-
cessing were not likely to be affected by bromocriptine treatment,
this finding strengthens the contention that hypertension in
PC2\(-/-\) mice is a reflection of impaired POMC processing
into \(\gamma\)-MSH. These observations provide further support for
an important role of \(\gamma\)-MSH in sodium metabolism; deficiency of
this peptide results in the development of salt-sensitive hyper-
tension.

The studies described above allow the development of a
scheme depicting the major elements of the physiology and
pathophysiology of the \(\gamma\)-MSH system in the regulation of
sodium metabolism (Fig. 5). Ingestion of a diet high in sodium
content generates a signal that ultimately reaches the pituitary.
The nature of this signal is at present obscure. It could arise
from sensory afferents in cardiopulmonary and arterial barore-
ceptor regions responding to the increase in blood volume,
which results from sodium retention in the early period of
exposure to the new dietary intake. An alteration in geometric
relationships of structures surrounding the third cerebral ven-
tricle has also been suggested to serve as a mediator of changes
in sodium intake (63), as has an increase in sodium concen-
tration in the cerebrospinal fluid (CSF) (82). This increase is
then transmitted to the pituitary NIL via a series of intermedi-
ate steps. We showed that the opioid receptor antagonist
naloxone blocked the postnephrectomy natriuresis when ad-
ministered directly into the CSF (68); insofar as pathways
regulating POMC processing and \(\gamma\)-MSH secretion in response
to a high dietary sodium intake are the same as those mediating
natriuresis after AUN, this observation indicates an opioidergic

![Fig. 5. Schematic diagram to indicate the role of \(\gamma\)-MSH in the adjustments to
an HSD. Through as yet unknown pathways, the HSD sends a signal to the
pituitary intermediate lobe to increase the mRNA abundance of POMC and its
processing enzymes PC1 and PC2. This leads to increased secretion of \(\gamma\)-MSH
and a rise in its concentration in plasma. The peptide then acts through its
melanocortin receptors (MC3-R), resulting in salt-sensitive hypertension. See
text for details.](image-url)
step in the neural pathway to the NIL. As discussed above, dopaminergic regulation of NIL function is established, and the data just presented indicate that manipulation of dopaminergic activity profoundly influences NIL γ-MSH content, plasma γ-MSH concentration, and MAP. Release of tonic dopaminergic suppression leads to increases in POMC and its processing enzymes PC1 and PC2 and an increase in NIL γ-MSH content. The NIL is viewed as the source of circulating γ-MSH (4, 44, 111), and plasma concentration of the peptide then rises. γ-MSH interacts with MC3-R in the kidney to stimulate natriuresis without an increase in GFR, indicating an inhibition of tubular reabsorption. Mice deficient in this receptor do not increase $U_{Na,V}$ during γ-MSH infusion, whereas γ-MSH-deficient PC2−/− mice show the expected natriuretic response to infusion of the peptide (88). The inhibition of sodium reabsorption by γ-MSH occurs via an interaction with some aspect of renal nerve function, because natriuresis does not occur in denervated kidneys (14). The high sodium intake must also activate other natriuretic pathways such as the ANP system and suppress antinatriuretic pathways like the renin-angiotensin system and aldosterone, all of which actions work in concert with γ-MSH to increase $U_{Na,V}$ to match the new dietary intake and thereby restore sodium balance. The natriuresis would contribute to the maintenance of normal blood pressure, although other actions of γ-MSH must also be involved. The lowering of MAP with γ-MSH infusion in γ-MSH-deficient PC2−/− mice with hypertension seems too rapid to be due solely to natriuresis, and the even more rapid reduction in MAP that occurs after intracerebroventricular administration of the peptide argues strongly for a central site of action. MC3-R are expressed in abundance in hypothalamus (1, 104), and γ-MSH does not lower MAP in hypertensive Mc3r-deficient mice (88). This would suggest that, in addition to stimulating NIL synthesis and secretion of the peptide, the HSD must also lead to central interaction of γ-MSH with MC3-R, probably in the hypothalamus, to maintain normal blood pressure. Much evidence links salt sensitivity of blood pressure to heightened sympathetic nervous system outflow (112), and it may be that γ-MSH, acting through MC3-R, serves as a tonic brake on sympathetic drive in the setting of excess dietary sodium intake. Failure of the synthesis and secretion of the peptide to increase during ingestion of the HSD, as in PC2−/− mice and bromocriptine-treated rats, or absent cell signaling as occurs in Mc3r−/− mice, releases this brake and allows sympathetic outflow to increase, resulting in salt-sensitive hypertension. If this increased outflow included increased efferent renal sympathetic nerve activity, sodium reabsorption would be stimulated (25). Such a scheme is of course highly speculative and will require extensive work to confirm. But it offers the opportunity to formulate discrete hypotheses that can be tested experimentally, extending our understanding of this previously unrecognized but functionally important hormonal system. Finally, it is worth pointing out the biphasic nature of γ-MSH injections or infusions identified by others (44, 73): in picomolar amounts the peptide is natriuretic and is antihypertensive in hypertensive rodents with γ-MSH deficiency, whereas in nanomolar-to-picomolar amounts natriuresis is lost and blood pressure and heart rate increase, reflecting stimulation of sympathetic outflow probably from central activation of the FM-RFamide gated sodium channel.

THE γ-MSH SYSTEM IN HUMANS

The work described above has indicated that γ-MSH derived from NIL POMC is importantly involved in the normal adjustments to a high sodium intake in rodents. In humans, no discrete intermediate lobe can be demonstrated anatomically, in distinction to rodents. However, cells can be identified in the human pituitary with histochemical and immunocytochemical characteristics of melanotrophs (2, 36, 44, 93, 128), and γ-MSH-like immunoreactivity has been identified, although not fully characterized, in human plasma (30, 31, 43, 118). Components of the γ-MSH system, including POMC, PC1, PC2, and various MC-Rs, are found in many human tissues (15, 127), and γ2-MSH has been extracted from human heart (31). The functional significance of these observations is not clear. Increases in plasma γ2-MSH concentration have been observed after graded exercise in young healthy subjects (118) and in patients with moderate-to-severe congestive heart failure (30). High-affinity binding of Lys-γ2-MSH to rat adrenal cortex has been reported (96), and Grif et al. (43) observed that plasma γ-MSH concentration was significantly elevated in patients with idiopathic hyperaldosteronism and aldosterone-producing adrenomas compared with patients with essential hypertension or normal subjects. Moreover, they found that Lys-γ2-MSH stimulated aldosterone secretion in a dose-dependent manner from dispersed cortical cells isolated from adrenal adrenomas removed from two patients (43). These observations opened the possibility that Lys-γ2-MSH could be a mediator of hyperaldosteronism-related hypertension, following up on the case study reported by Franco-Saenz and colleagues (36). However, the significance of these findings must be questioned after the cloning and characterization of the melanocortin receptors has revealed that MC2-R, expressed predominately in the adrenal cortex, has affinity only for ACTH, with no appreciable affinity for the MSH peptides (108). Messenger RNA for MC5-R has also been detected in low abundance in human adrenal gland (15), so it is possible that signaling by MSH peptides could occur via this receptor. This also seems unlikely given that the affinity of this receptor for γ-MSH peptides is very low (109).

Mutations in genes of the melanocortin system have been observed in humans but to date have not shed light on the role of this system in cardiovascular homeostasis. Two children with genetic POMC deficiency have been described (60). They had adrenal insufficiency because of lack of ACTH, red hair pigmentation because of α-MSH deficiency, and obesity. With glucocorticoid replacement, they developed normally but remained obese. Cardiovascular function in these patients was reported as normal (61). The phenotype of these children is duplicated in Pomc null mice (129). Mutations in melanocortin receptors have also been identified as causes of obesity. Screening of an obese population detected mutations in MC4-R in 4% of the subjects, suggesting that such mutations might be relatively common genetic causes of obesity (117). Severity of the obesity correlated with the in vitro activity of the mutant MC4-R (34) and binge eating has been identified as a phenotype of MC4-R gene mutations (9). As mentioned earlier, targeted disruption of mouse Mc4r results in an obese phenotype with normal blood pressure. Mutations in MC3-R have also been identified, but attempts to link them to obesity have been unsuccessful (64, 66, 106). There is virtually no information
about this system, particularly γ-MSH, in cardiovascular homeostasis in humans. It is to be hoped that the data summarized in this review will stimulate interest in the study of this area.

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