Systemic α-melanocyte-stimulating hormone administration decreases arthritis-induced anorexia and muscle wasting

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Gómez-SanMiguel AB, Martín AI, Nieto-Bona MP, Fernández-Galaz C, López-Menduiña M, Villanúa MA, López-Calderón A. Systemic α-melanocyte-stimulating hormone administration decreases arthritis-induced anorexia and muscle wasting. Am J Physiol Regul Integr Comp Physiol 304: R877–R886, 2013. First published March 20, 2013; doi:10.1152/ajpregu.00447.2012.—Rheumatoid cachexia is associated with rheumatoid arthritis and it increases mortality and morbidity. Adjuvant-induced arthritis is an experimental model of rheumatoid arthritis that causes anorexia and muscle wasting. α-Melanocyte-stimulating hormone (α-MSH) has anti-inflammatory actions, and it is able to decrease inflammation in several inflammatory diseases including experimental arthritis. In this study we tested whether systemic α-MSH treatment is able to ameliorate cachexia in arthritic rats. On day 8 after adjuvant injection control and arthritic rats were treated with α-MSH (50 μg/rat ip) twice a day, until day 16 when all rats were euthanized. Arthritis decreased food intake, but it increased hypothalamic expression of neuropeptide Y (NPY) and Agouti-related peptides (AgRP) as well as interleukin-1β (IL-1β) and cyclooxygenase-2 (COX-2) mRNA. In arthritic rats, α-MSH decreased the external signs of arthritis and increased food intake (P < 0.01). In addition, α-MSH decreased hypothalamic expression of IL-1β, COX-2, proopiomelanocortin, and prohormone-converting (PC) enzymes PC1/3 and PC2 mRNA in arthritic rats. In control rats, α-MSH did not modify food intake or hypothalamic expression of aforementioned mRNA. α-MSH prevented arthritis-induced increase in gastrocnemius COX-2, muscle-specific RING-finger protein-1 (MuRF1), and atrogin-1 expression, and it increased fast myofiber size. In conclusion our data show that in arthritic rats peripheral α-MSH treatment has an anti-cachectic action increasing food intake and decreasing muscle wasting.

ADJUVANT-INDUCED ARTHRITIS is an experimental model of rheumatoid arthritis that can be induced in rats by an intradermal injection of Freund’s adjuvant and is associated with cachexia. Ten days after adjuvant injection rats develop the external signs of arthritis and stop gaining weight (6). Although adjuvant arthritis induces anorexia (29), body weight loss is not only due to lower food intake, since body weight gain in arthritic rats was lower than in pair-fed rats (6). The decrease in body weight in arthritic rats is associated with muscle wasting (6, 42). Muscle wasting in chronic illnesses is secondary to an increase in the activity of the ubiquitin-proteasome proteolytic pathway (17, 38). Two E3 ubiquitin ligases, muscle-specific RING-finger protein-1 (MuRF1) and atrogin-1, are the key enzymes in this process. These E3 ubiquitin-ligating enzyme genes, called atrogenes, are sensitive markers for muscular atrophy (4) and they are increased in the skeletal muscle of arthritic rats (22).

Melanocortins (α-, β-, and γ-MSH) are peptides derived from proopiomelanocortin (POMC) that have potent anti-inflammatory activity both in vivo and in vitro (for review see Ref. 5). These peptides are formed following posttranslational processing of POMC by prohormone-converting (PC) enzymes PC1/3 and PC2. PC1/3 produces ACTH and β-lipotropin (β-LPH) and β-endorphin from POMC. These fragments are additionally processed by PC2 to release smaller fragments: α-MSH from ACTH, β-MSH from γ-lipotropin (γ-LPH), and γ-MSH from N-POMC. The peptide α-MSH is able to decrease pro-inflammatory cytokine release after endotoxin administration (46) and in immune cell cultures (67). Melanocortins also exert their effects at the end of the inflammatory process, increasing the levels of anti-inflammatory cytokines (52, 55) and the resolution of the inflammatory response (43). α-MSH has been shown to ameliorate the course of chronic inflammatory illnesses in experimental animals such as autoimmune encephalomyelitis (68) and inflammatory bowel disease (53). Treatment with α-MSH is also able to inhibit inflammation in experimental arthritis (9, 43), and it has been postulated as a potential therapeutic target for the treatment of arthritic pathologies (18).

On the contrary, a possible use for melanocortin antagonist in reversing cachexia in human disease has been proposed (41). This hypothesis was based on the fact that α-MSH plays an important role in the control of energy balance in the brain. In the arcuate nucleus of the hypothalamus there are two classes of neurons involved in food intake regulation (for review see Refs. 62 and 70). One of them is the anorexigenic neurons that coexpress POMC and cocaine-amphetamine-regulated transcript (CART). These neurons release α-MSH, which inhibits food intake (13). The second subset of arcuate neurons expresses orexigenic peptides, neuropeptide Y (NPY) and Agouti-related protein (AgRP). AgRP is the endogenous antagonnist of MSH receptors (MCR), thereby antagonizing the anorexigenic effects of α-MSH (14). There are several data indicating that blockade of MSH receptor-4 (MC4-R) attenuates anorexia and cachexia in rodent models of cancer, renal failure, and heart failure (11, 40, 60). However, there is also evidence that MC4-R is involved in the anti-inflammatory effect of α-MSH. Peripheral administration of MC4-R agonist has a protective effect against hypoxic-ischemic injury (24, 44, 63).
Taking into account the potent anti-inflammatory effects of α-MSH, we hypothesize that systemic α-MSH administration to arthritic rats may inhibit muscle expression of ubiquitin ligases MuRF-1 and atrogin-1, as well as skeletal muscle wasting, resulting in amelioration of cachexia. However, as mentioned above, in the brain α-MSH decreases food; therefore, the effect of systemic α-MSH treatment on food intake and hypothalamic expression of neuropeptides was also studied.

MATERIALS AND METHODS

Arthritic and control male Wistar rats were purchased from Charles River (Barcelona, Spain). Arthritis was induced in the isoflurane-anesthetized rats by an intradermal injection of 4 mg heat-inactivated Mycobacterium butyricum in the right hindpaw (6). Control animals were injected with vehicle (0.1 ml of paraffin oil). After the rats arrived to the lab (day 3 after adjuvant injection), rats were housed 3–4 per cage under controlled conditions of temperature (22 °C) and light (lights on from 7:30 to 19:30 h). Food and water were available ad libitum. All procedures on animals were carried out according to the procedures following the guidelines recommended by the European Union for the care and use of laboratory animals and were approved by the Complutense University Animal Care Committee.

Experimental protocol. On day 8 after adjuvant injection, both control and arthritic rats were randomly divided into two groups, each with 10 rats. One group was treated with 250 μl ip of saline, twice a day (at 9:00 AM and at 5:00 PM). The second group received similar treatment with 50 μl/rat of α-MSH trifluoroacetate salt (Bachem, Bubendorf, Switzerland) dissolved in saline. At this dose α-MSH is able to decrease clinical signs of experimental arthritis in rats (9). As arthritis decreases food intake, a pair-fed group injected with saline was also included. Pair-fed rats received the same amount of food (g/100 g body wt) eaten on the previous day by arthritic rats treated with saline. Assessment of arthritis was performed by measuring the arthritis index of each animal, which was clinically scored by grading each paw from 0 to 4. Grading was determined as the following: 0, no erythema or swelling; 1, slight erythema or swelling of one or more digits; 2, swelling of paw; 3, swelling of entire paw and ankle; and 4, ankylosis, incapacity to bend the ankle. The severity score was the sum of the clinical scores of each limb, the maximum value being 16.

Body weight, food intake, and arthritis index scores were examined daily. Food intake per cage was calculated by measuring the difference between the initial and the remaining amount of pellets in the feeder and expressed as gram per 100 g body wt per day. All rats were euthanized by decapitation 16 days after adjuvant injection and after 8 days of α-MSH treatment, 2.5 h after the last injection. Trunk blood was collected in cooled tubes, allowed to clot, and centrifuged. The serum was stored at −20 °C until leptin assay was performed.

Immediately after decapitation, left gastrocnemius muscles were removed, dissected, weighed, and frozen in liquid nitrogen and stored at −80°C until RNA or protein extraction. The medial basal hypothalami were dissected as previously described (37), quickly frozen in liquid nitrogen, and stored at −80°C for isolation of RNA. Isolation and manipulation of tissues were always performed under sterile conditions.

Table 1. Primers for real-time PCR

<table>
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<th>Gene</th>
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<th>Reverse Primer (5’ to 3’)</th>
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See text for abbreviation definitions.

Fig. 1. Treatment with α-melanocyte-stimulating hormone (α-MSH; 50 μl/rat twice daily) decreased arthritis score (A) and increased body weight gain (B) in arthritic rats. C, control rats; AA, arthritic rats; PF, pair-fed rats. Data represent means ± SE (n = 9–10). **P < 0.01, #P < 0.05 vs. pair-fed rats, °°P < 0.01, °P < 0.05 vs. arthritic rats treated with saline.
Muscle fiber cross-sectional size was measured as an index of fiber atrophy. The extracellular matrix was detected by wheat germ agglutinin (WGA), labeled with Texas Red (1 μg/ml; W849, Invitrogen), and slow muscle fibers were detected with a monoclonal antibody against slow myosin heavy chain form (1:80, NCL-MHCs-Novocastra; Newcastle upon Tyne, UK) and secondary Alexa fluor 488 Goat antimouse IgG (1:100, A11001-Invitrogen; Invitrogen, Madrid, Spain). Sections were mounted with Prolong-Gold antifade reagent.

RNA extraction and real-time PCR. Hypothalami and gastrocnemius muscle (100 mg) were homogenized, and total RNA was extracted using Ultraspec (Biotec Laboratories, Houston, TX), following the manufacturer’s protocol. The final concentration of RNA was determined with a BioPhotometer (Eppendorf, Germany), and the integrity of the RNA was confirmed by agarose gel electrophoresis. First-strand cDNA synthesis was performed using 1 μg of total RNA with Quantscript Reverse Transcription kit (Qiagen CombH Hilden, Valencia, CA). Primers for real-time PCR (Table 1) were obtained from Roche (Madrid, Spain) by using the EXIQON Universal Probe Library. The thermal cycling profile consisted of a preincubation step at 95°C for 10 s followed by 40 cycles of 95°C denaturation steps for 15 s, 60°C annealing steps for 30 s, and 72°C extension steps for 30 s. Results were expressed relative to the control animals treated with saline, where the relative mRNA abundance has been arbitrarily set to 1, using cycle threshold 2 (ΔΔCT) method, with 18S as reference gene. PCR products were separated using agarose gel electrophoresis to confirm product presence and size.

Gastrocnemius morphology. Left gastrocnemius were dissected and weighed. For immunohistochemical studies the medial part of the left gastrocnemius was placed on a transparency film, glued at one end to a cork with gum tragacanth (Fibraguar, Fardi, Madrid, Spain), frozen in isopentane, cooled by liquid nitrogen, and stored at −80°C. Cryostat sections of 10 μm were fixed with 100% acetone and stained with hematoxylin-eosin. Parallel sections were kept at −80°C until further processing for immunohistochemical analysis. Sections were scanned (Epson scanner 4990) with a transparent rule and the area was measured with Image J software.

Fig. 2. Effect of arthritis and α-MSH (50 μg/rat twice daily) on daily food intake (A) and serum concentration of leptin (B). Arthritis decreased food intake (P < 0.01). α-MSH administration increased food intake in AA rats (P < 0.01) but not in control rats. PF rats had lower serum concentrations of leptin than control rats (P < 0.01) but higher than AA rats (P < 0.05). Data represent means ± SE (n = 8–10). Values without the same letter are significantly different.

Fig. 3. Effect of arthritis and α-MSH (50 μg/rat twice daily) on hypothalamic cytokine mRNA fold increases (A) and IL-10 (C) mRNA fold increase. mRNA was quantified using real-time PCR and is presented in relation to the mean value in control group. Arthritis increased hypothalamic COX-2 and IL-1β (P < 0.01) mRNA fold increase. Data represent means ± SE (n = 5–8 rats). Values without the same letter are significantly different.
combined with DAPI (P36931, Invitrogen). Digital images were acquired with a Leica DMi3000 microscope. Fiber boundaries were detected from WGA fluorescent images using Difference of Gaussians algorithm by GIMP software. According to the distribution of the slow fibers, two zones were differentiated on each section, one zone with only fast fibers and one mixed zone with slow and fast fibers. A separate measurement of fast and slow fiber areas was done respectively on each zone with ImageJ software. The number of fibers measured on each zone and section oscillated between 400 and 700.

**Immunoblot.** Muscle samples were homogenized in lysis buffer (10 μl/mg) with protease inhibitor cocktail (Sigma-Aldrich, Madrid, Spain). The homogenate was later centrifuged at 13,000 rpm at 4°C for 30 min to remove tissue debris. Protein concentration was determined using the Bradford protein assay with bovine serum albumin as standard. The protein extract was boiled for 5 min with a 1:1 volume of Laemmli loading buffer. Proteins (50 μg) were resolved by electrophoresis on 15% polyacrylamide gels under reducing conditions and then transferred onto PVDF membranes that were blocked by Tris-buffered saline. Membranes were probed overnight at 4°C sequentially with antibodies against cyclooxygenase-2 (COX-2, Cayman Chemical, Ann Arbor, MI) or MuRF1 (Santa Cruz Biotechnology, Santa Cruz, CA) and α-tubulin (Sigma-Aldrich) with stripping of membranes before each new antibody. Membranes were then incubated for 90 min in the appropriate horseradish peroxidase-conjugated secondary antibody (anti-mouse IgG Amersham Biosciences, Little Chalfont, UK; anti-rabbit IgG Bio-Rad, Madrid, Spain), and peroxidase activity was detected using enhanced chemiluminescent reagent (Amersham Biosciences). Band intensities were quantified by densitometry using Gene Tools Analysis software. The density of the protein band in each lane was expressed as the percentage of the mean density of control rats, after loading normalization using α-tubulin.

**Serum leptin measurement.** Serum concentrations of rat leptin were determined by radioimmunoassay using a commercial kit from LINCO Research (St. Charles, MO) following the manufacturer’s protocol.

**Statistical analysis.** Statistics were computed using the statistics program STATGRAPHICS plus for Windows. Data are presented as means ± SE and were tested with ANOVA; post hoc comparisons were made using the LSD multiple range test. Statistical significance was set at P < 0.05.

**RESULTS**

In arthritic rats injected with saline the external signs of inflammation increased daily starting on day 9 after adjuvant injection, reaching its maximum value on day 16. In arthritic rats injected with α-MSH evolution of arthritis scores increased parallel to those of arthritic rats injected with saline until day 12. After that arthritis scores stopped increasing, having similar values between days 12 and 16 (Fig. 1A).

Arthritis and pair-feeding the rats decreased body weight gain (Fig. 1B). Arthritic rats injected with saline had lower body weight values between days 12 and 16 than on day 8. In arthritic rats the decrease in body weight gain was not only due to the decrease in food intake, since body weight gain in pair-fed rats was higher than in arthritic rats injected with saline from day 10 to 16. α-MSH treatment did not modify body weight in control rats (Fig. 1B). On the contrary, arthritic rats treated with α-MSH had higher body weight gain than arthritic rats treated with saline and was similar to pair-fed rats between days 14 and 16.

Arthritis decreased daily food intake (P < 0.01, Fig. 2A). α-MSH treatment did not modify food intake in control rats, whereas in arthritic rats α-MSH increased food intake (P < 0.01). As shown in Fig. 2B, both arthritis and pair feeding the rats decreased serum concentrations of leptin (P < 0.01). However, arthritic rats had lower leptin levels than pair-fed rats (P < 0.01). α-MSH treatment did not modify serum concentrations of leptin in control or in arthritic rats.

Arthritis induced a significant increase (P < 0.01) in hypothalamic COX-2 and IL-1β mRNA in rats treated with saline, whereas this increase was prevented by α-MSH administration to arthritic rats (Fig. 3, A and B). In the rats treated with α-MSH, arthritis increased hypothalamic expression of IL-10 to levels higher than in control and pair-fed rats (P < 0.01), but in the rats treated with saline this increase was not significant (Fig. 3C).

Arthritis increased hypothalamic NPY expression (P < 0.01), whereas α-MSH administration and pair feeding the rats did not modify NPY mRNA (Fig. 4A). Arthritis also increased AgRP expression in the hypothalamus (P < 0.01, Fig. 4B). Neither pair feeding the rats nor α-MSH treatment to control rats modified AgRP levels. However, arthritic rats treated with α-MSH had lower hypothalamic AgRP mRNA than arthritic rats treated with saline but higher than pair-fed rats.

Pair-fed rats had lower POMC mRNA levels than control rats treated with saline (P < 0.05, Fig. 5A). In contrast, arthritic rats treated with saline had higher POMC expression than pair-fed rats (P < 0.05) and similar to control rats. α-MSH administration did not modify POMC expression in control rats, but it decreased POMC mRNA in arthritic rats (P < 0.05) to levels similar to those observed in pair-fed rats. Hypothe-
Arthritic rats had lower gastrocnemius weight than pair-fed rats (545 ± 30 mg, means ± SE vs. 1,034 ± 25 mg, P < 0.01). α-MSH administration to arthritic rats increased gastrocnemius weight (631 ± 21 mg, means ± SE, P < 0.05). Arthritic rats had lower (P < 0.01) mean fast fiber cross-sectional area (CSA) than pair-fed rats (Fig. 6, A and C). Administration of α-MSH to arthritic rats increased mean fast fiber CSA (P < 0.05), but their values were lower than those of pair-fed rats. Arthritic rats also decreased mean slow fiber CSA (P < 0.01, Fig. 6B), but α-MSH treatment was not able to increase the mean slow fiber CSA.

COX-2 levels in the gastrocnemius were significantly increased (P < 0.01) in arthritic rats treated with saline (Fig. 7). Treatment with α-MSH reduced gastrocnemius COX-2 in arthritic rats to levels similar to those of control and pair-fed rats. As expected, arthritic rats treated with saline had higher MuRF1 mRNA and protein (P < 0.01, Fig. 8, A and C). α-MSH administration to arthritic rats totally prevented the stimulatory effect of arthritis on MuRF1 in the gastrocnemius muscle. Arthritis also increased (P < 0.01) atrogin-1 mRNA in the gastrocnemius (Fig. 8C). In arthritic rats α-MSH treatment decreased atrogin-1 mRNA (P < 0.01) but to levels higher than those of control and pair-fed rats (P < 0.05).

DISCUSSION

Our data show that the anti-inflammatory effect of peripheral α-MSH administration to arthritic rats is associated with a decrease in arthritis-induced anorexia, body weight loss, and hypothalamic IL-1β, COX-2, POMC, PC1/3, and PC2 mRNA. In addition, α-MSH prevents arthritis-induced upregulation of the ubiquitin-proteasome ligases atrogin-1 and MuRF1 in the skeletal muscle.

As previously reported (9, 43), α-MSH administration has an anti-inflammatory effect in arthritic rats. It decreased the external signs of arthritis and prevented arthritis-induced expression of COX-2 in the gastrocnemius. Systemically administered, α-MSH was also able to decrease COX-2 and IL-1β, whereas it increased IL-10 expression in the hypothalamus. The ability of systemically injected α-MSH to interfere with the inflammatory process in the brain by inhibiting cytokine production has been previously reported after intracerebroventricular administration of endotoxin (54).

Pair feeding the rats decreased hypothalamic POMC mRNA levels, whereas those of NPY and AgRP were not modified. A decrease in arcuate POMC levels with no differences in arcuate NPY and AgRP mRNA has also been reported in food-restricted rats for 4 wk (32). The fact that arthritic rats had POMC mRNA levels similar to control rats, although their food intake was decreased, indicates that there is some factor that upregulated or prevented the decrease in POMC mRNA induced by the lower food intake. Although arthritis decreased food intake, we found that arthritis increased the expression of hypothalamic orexigenic peptides NPY and AgRP. Similar data have been reported by Stofkova et al. (54, 65). Upregulation of orexigenic peptides also occurs in another illness that induces anorexia and cachexia such as cancer (30, 35, 47, 66). Those changes suggest that physiological response in the arcuate nucleus of the hypothalamus might combat rather than mediate arthritis-induced anorexia and cachexia.

Fig. 5. Effect of arthritis and α-MSH (50 μg/rat twice daily) on hypothalamic proopiomelanocortin (POMC) (A), PC1 (B), and PC2 mRNA (C). mRNA was quantified using real-time PCR and is presented in relation to the mean value in control group. α-MSH administration decreased hypothalamic expression of POMC, PC1, and PC2 mRNA in AA rats (P < 0.05). Data represent means ± SE (n = 7–10 rats). Values without the same letter are significantly different.

Hypothalamic CART expression was not significantly modified by arthritis or α-MSH administration (data not shown). Arthritis increased hypothalamic PC2 mRNA levels (P < 0.01) but not those of PC1 (Fig. 5, B and C). α-MSH treatment did not modify hypothalamic PC1 or PC2 mRNA in control rats. However, in arthritic rats α-MSH decreased all these mRNA to levels similar to those of pair-fed rats. Although we do not know how these changes contribute to POMC processing, α-MSH possibly decreased hypothalamic biosynthesis and processing of POMC in arthritic rats.
Hypothalamic response to chronic cachectic states is the opposite of that observed after acute inflammatory stimuli, where an increase in the expression of anorexigenic peptides together with a decreased expression of orexigenic peptides has been reported (61). Another difference between acute and chronic inflammation is serum leptin levels. Serum concentration of leptin is increased after acute inflammation induced by endotoxin administration (16, 25). On the contrary, leptin levels are decreased by chronic LPS administration (25) or by chronic inflammatory illnesses such as cancer (19) or experimental arthritis (21, 64). Leptin is one of the main regulators of hypothalamic NPY and AgRP, and it decreases their expression (33). Accordingly, the decrease in serum concentrations of leptin in arthritic rats can be responsible for the increased expression of anorexigenic hypothalamic peptides. In this sense, the opposite response has been reported in obese rats, where food intake and circulating leptin is increased although POMC is increased and NPY is reduced (14, 20).

Prohormone convertases PC1/3 and PC2 are expressed in endocrine and neuroendocrine cells and they are important in prohormone processing (48). PC1/3 and PC2 are posttranslational POMC processing enzymes that lead to melanocortins. Hypothalamic POMC neurons have ObR, and leptin has been shown to upregulate hypothalamic POMC, PC1/3, and PC2 levels leading to coordinated processing of prohormones into mature peptides (58). On the contrary, fasting decreases circulating leptin, hypothalamic POMC, MSH, and PC1, whereas leptin administration reverses these effects (49, 58). It has been proposed that cancer-induced anorexia and cachexia can be caused by activation of hypothalamic POMC neurons by a leptin-independent mechanism (61). In our data, circulating leptin levels were reduced both by arthritis and by pair feeding the rats, but only pair feeding decreased hypothalamic POMC expression. In addition to POMC, arthritic rats had higher PC1/3 and PC2 than pair-fed rats, and this difference disappeared when arthritic rats were treated with α-MSH. We can speculate that the decrease in POMC, PC1, and PC2 in arthritic rats treated with α-MSH may contribute to their attenuated anorexia and

Fig. 6. Mean cross-sectional area (CSA) of fast (A) and slow (B) myofibers of the gastrocnemius in pair-fed (PF) and in AA rats treated with saline or α-MSH (50 µg/rat twice daily). Representative transverse sections from fast fiber region of gastrocnemius muscle (C). All fibers are outlined by wheat germ agglutinin (white). Nuclei were tagged using DAPI. Bar = 50 µm. Arthritis decreased gastrocnemius fast and slow fiber cross-sectional area (P < 0.01). α-MSH increased fast fiber size in AA rats (P < 0.05). Data represent means ± SE (n = 4–6). Values without the same letter are significantly different.

Fig. 7. Effect of arthritis and α-MSH treatment (50 µg/rat twice daily) on gastrocnemius COX-2. Arthritis increased COX-2 in the gastrocnemius of rats treated with saline (P < 0.01) but not in those treated with α-MSH. COX-2 was measured by Western blotting, normalized against α-tubulin, and expressed as percentage of the control rats. Data represent means ± SE (n = 8–10). Values without the same letter are significantly different.
weight loss. However, a limitation of the current study is that the observed changes in hypothalamic gene expression may not precisely reflect changes at protein levels. Unfortunately, the sample size limitation in protein analysis should be recognized. Further studies are required to extend these results to protein activity or signaling pathways to confirm the suspected mechanisms discussed here.

The lack of a direct anorexigenic effect of systemic α-MSH administration could be explained by the difficulty in crossing the blood-brain barrier. These data suggest that in arthritic rats treated with α-MSH the attenuated anorexigenic response can be secondary to the anti-inflammatory effect of α-MSH. Anorexia during systemic inflammation seems to be mainly dependent on increases in IL-1β and COX-2 expressions in the hypothalamus. Therefore, increased expression of hypothalamic IL-1β and COX-2 can mediate the anorexigenic effect of arthritis. IL-1β induces a dose-dependent decrease in food intake (15, 54, 50). Increased hypothalamic expression of IL-1β has been reported in other illnesses associated with cachexia and anorexia such as cancer (25, 51). In addition, central IL-1β blockade is able to prevent LPS-induced anorexia (34). COX-2 is strongly induced in brain vasculature by IL-1β (8) and subsequently synthesizes and releases prostaglandin E2 that diffuses into the brain parenchyma and elicits inflammation-associated anorexia (31). Furthermore, COX-2 inhibition had no appreciable effect on food intake in control rats but induced a marked increase in food intake in arthritic rats (23). Therefore, the inhibitory effect of α-MSH treatment on hypothalamic IL-1β and COX-2 can be one of the mechanisms through which α-MSH increases food intake in arthritic rats. POMC neurons of the ARC have IL-1β receptors, and IL-1β induced the expression of Fos protein in ARC POMC neurons and MSH release from hypothalamic explants (59). COX-2 activation is also involved in endotoxin-induced decrease in food intake and activation of hypothalamic POMC neurons (57). In arthritic rats α-MSH administration increased the anti-inflammatory cytokine IL-10. The stimulatory effect of α-MSH on IL-10 is well known (2, 10, 71). Furthermore, it has been reported that central IL-10 administration attenuates the decrease in food intake and in body weight induced by acute inflammation (3, 27, 36). When it is taken into account that systemic α-MSH is able to modulate the production of brain cytokines and prostaglandins and to affect appetite controlling systems in arthritic rats, the effects of α-MSH treatment in decreasing hypothalamic inflammation and increasing food intake seem to be related.
As we have previously reported (7), in arthritic rats the decrease in gastrocnemius weight is associated with a reduction in cross-sectional size of both fiber types, fast and slow twitch, where the atrophy is higher in fast twitch fibers. Similarly, in other states that induced cachexia, such as cancer and sepsis, fast fibers are also more atrophied than slow fibers (1, 69). The stimulating effect of α-MSH on food intake in arthritic rats is associated with an increase in body weight gain and in cross-sectional area of the fast myofiber. The beneficial action of α-MSH in the skeletal muscle is also evidenced by the ability of α-MSH to prevent arthritis-induced upregulation of two genes of the ubiquitin-proteasome system MuRF1 and atrogin-1, as well as COX-2 increase in muscle. We have previously reported that COX-2 inhibition, by meloxicam administration to arthritic rats, decreases skeletal muscle wasting by blocking upregulation of muscle atrogin-1 and MuRF1 (23). Celecoxib, another COX-2 inhibitor, has been reported to prevent cachexia induced by an experimental model of arthritis plus atherosclerosis (56). In addition, COX-2 inhibitors are also able to improve cancer cachexia in both experimental animals and in humans (12, 28, 39), by acting on the ubiquitin-proteasome pathway (26). All these data suggest that the effect of α-MSH on muscle COX-2 can be one of the mechanisms through which α-MSH prevents MuRF1 and atrogin-1 activation and ameliorates muscle wasting.

In conclusion, our data show that in arthritic rats peripheral α-MSH treatment has an anti-cachectic action increasing food intake and decreasing muscle wasting.

**Perspectives and Significance**

Rheumatoid cachexia is an important contributor in increasing morbidity and premature mortality in rheumatoid arthritis patients. Adjuvant-induced arthritis in rats is a well-established model of rheumatoid arthritis that is associated with anorexia and muscle wasting secondary to an increase in the activity of the ubiquitin-proteasome proteolytic pathway. α-MSH is a neuropeptide that has anorexigenic and anti-inflammatory effects. Our data show that systemic α-MSH administration decreases arthritis-induced anorexia, hypothalamic inflammation, and POMC expression. α-MSH also decreases the external signs of arthritis, increases body weight gain, and prevents upregulation of COX-2 and atrogens in the gastrocnemius muscle of arthritic rats. The pathways through which α-MSH attenuates inflammation, increases food intake, and decreases muscle wasting should be clarified by future studies analyzing the possible melanocortin receptors involved as well as the site of action. Our data suggest that α-MSH and/or melanocortin receptor agonists are potential therapeutic strategies in inhibiting inflammatory responses and preserving food intake, body weight, and muscle mass in chronic inflammatory illnesses such as rheumatoid arthritis.

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**DISCLOSURES**

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