

Plasma α -Melanocyte Stimulating Hormone Predicts Outcome in Ischemic Stroke

Dannielle Zierath, BS; Pat Tanzi, BSN, RN, CCRC; Kevin Cain, PhD;
Dean Shibata, MD; Kyra Becker, MD

Background and Purpose— α -Melanocyte stimulating hormone (α -MSH) is an endogenously produced neuropeptide derived from the same precursor as adrenocorticotrophic hormone. α -MSH has profound immunomodulatory properties and may also be neuroprotective. Nothing is known about α -MSH and changes in its plasma concentrations in patients with acute ischemic stroke.

Methods—In this prospective observational study, plasma concentrations of α -MSH, adrenocorticotrophic hormone, cortisol, and interleukin 6 were assessed longitudinally over the course of 1 year after stroke onset in 111 patients. Logistic regression was used to the effect of initial plasma α -MSH, adrenocorticotrophic hormone, cortisol, and interleukin 6 on long-term outcome.

Results—There was an early decrease in plasma α -MSH in patients with severe stroke (National Institutes of Health Stroke Scale ≥ 17) that normalized over the course of the year; these same patients evidenced elevations in plasma cortisol and interleukin 6. Higher initial plasma α -MSH, but not adrenocorticotrophic hormone, cortisol, or interleukin 6, was independently predictive of good long-term outcome.

Conclusions—This research is the first to study endogenous changes in plasma α -MSH after stroke. The independent effect of early plasma α -MSH on stroke outcome, as well as a growing body of experimental data demonstrating improved stroke outcome with exogenous α -MSH administration, suggests a potential therapeutic role for α -MSH in the treatment of stroke. (*Stroke*. 2011;42:3415-3420.)

Key Words: α -MSH ■ stroke ■ outcome

Alpha-melanocyte stimulating hormone (α -MSH) is a 13 amino acid neuropeptide derived from proopiomelanocortin (POMC), a prohormone polypeptide expressed in the brain, the pituitary gland, and in peripheral tissues, such as the immune system and skin.¹ The processing of POMC depends on a series of prohormone convertases (PCs), and the tissue in which POMC is processed determines its eventual end products. In brain, POMC is expressed in the arcuate nucleus of the hypothalamus. Cleavage by PC1 leads to the production of adrenocorticotrophic hormone (ACTH) and β -lipotropin; further processing by PC2 leads to production of smaller peptides, including α -MSH and β -endorphin.^{1,2} α -MSH production is, thus, more robust in tissues that highly express PC2.

As part of the acute phase/stress response in stroke, cortisol is elevated, and these elevations correlate with both stroke severity and outcome.³⁻⁵ Cortisol production depends largely on the expression of ACTH, and given the common origin of ACTH and α -MSH, we hypothesized that there might be stroke-induced alterations in α -MSH. In patients with traumatic brain injury, for instance, decreases in plasma α -MSH occur and are associated with worse outcome.⁶ To our knowledge, no studies have evaluated endogenous changes in α -MSH after stroke. Based on

experimental data, however, a decrease similar to that seen in patients with traumatic brain injury is anticipated.⁷ We, thus, sought to describe the time course of changes in plasma α -MSH after ischemic stroke in relation to ACTH and cortisol. Because not all cortisol production depends on ACTH, however, we also assessed plasma interleukin (IL) 6, a cytokine that activates the hypothalamic-pituitary-adrenal axis and can directly stimulate cortisol production by the adrenal gland.⁸

Methods

Research Subjects

This study was part of a larger prospective study that followed immune responses over the course of the year after stroke onset.^{9,10} The study was approved by the institutional review board, and all of the patients or their surrogates provided informed consent. Patients with ischemic stroke admitted to either Harborview Medical Center or the University of Washington Medical Center from September 2005 through May 2009 who were ≥ 18 years of age could be enrolled within 72 hours of symptom onset and were felt not likely to die from their stroke were eligible. Patients with ongoing therapy for malignancy, known history of HIV or hepatitis B or C, history of brain tumor, anemia (hematocrit < 35 on admission), and those taking immunomodulatory drugs were excluded. Blood was drawn

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From the Departments of Neurology (D.Z., P.T., K.B.), Biostatistics (K.C.), and Radiology (D.S.), University of Washington School of Medicine, Harborview Medical Center, Seattle, WA.

Correspondence to Kyra Becker, Box 359775, Harborview Medical Center, 325 9th Ave, Seattle, WA 98104-2499. E-mail kjb@uw.edu

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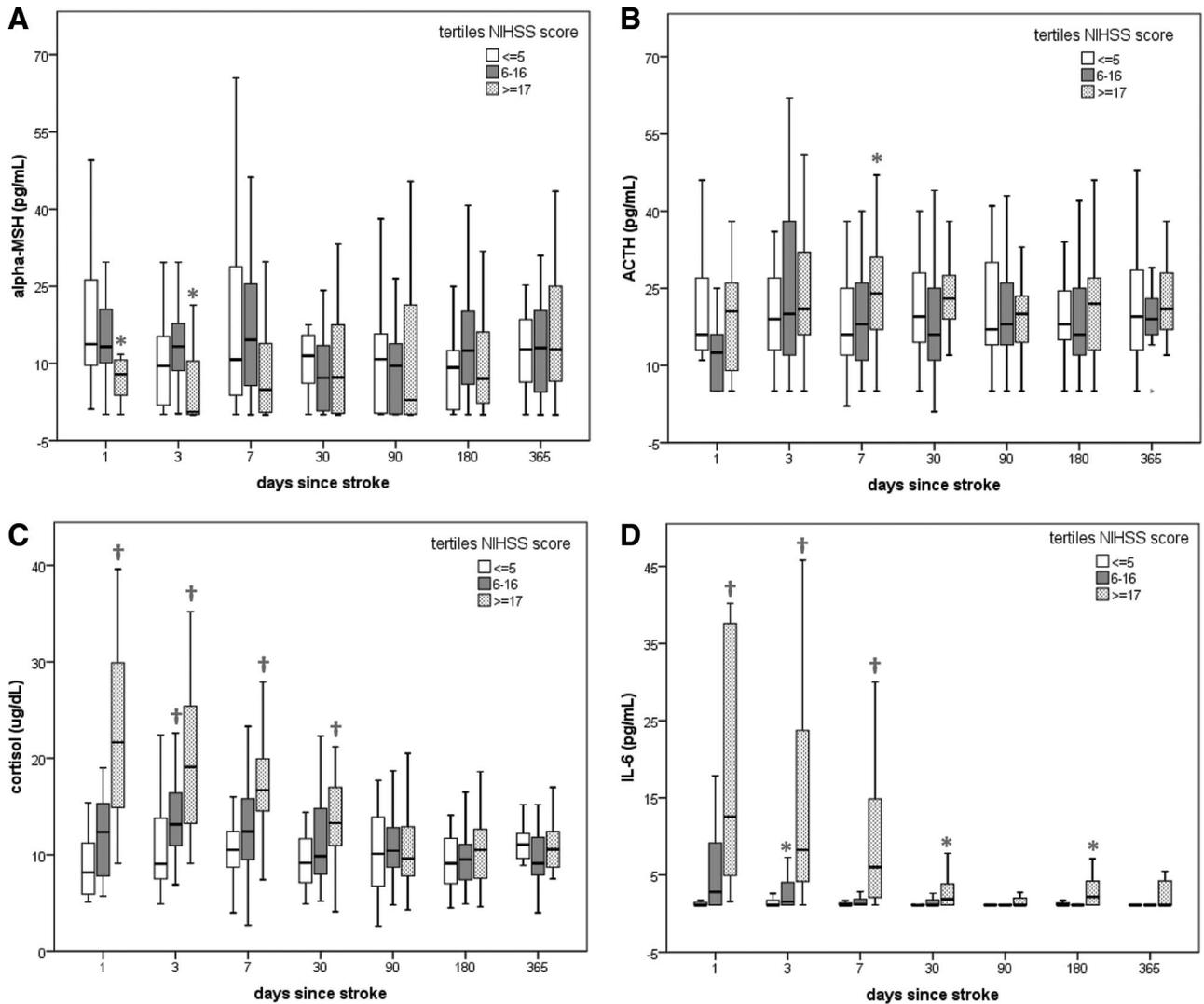


Figure. Plasma concentrations of α -melanocyte stimulating hormone (α -MSH; **A**), adrenocorticotrophic hormone (ACTH; **B**), cortisol (**C**), and interleukin (IL) 6 (**D**) over the course of 1 year after stroke. Box plots depict the median and interquartile range. Data are depicted by tertile of stroke severity, differs from the lowest tertile by * $P \leq 0.05$ or † $P \leq 0.001$.

as soon as possible after stroke onset and at 3, 7, 30, 90, 180, and 365 days after stroke onset. Plasma was frozen at -80° until use.

Clinical Data

Demographic and clinical data were collected on all of the patients. Stroke severity was determined by the National Institutes of Health Stroke Scale score and outcome by the modified Rankin Scale. Total infarct volume on initial diffusion-weighted MRI was calculated by the ABC/2 method by a single radiologist trained in the Cardiovascular Health Study and Atherosclerosis Risk in Communities protocols for infarct scoring.¹¹ Information about therapeutic interventions for the treatment of stroke and stroke-related complications, such as infection, was collected. Infection was defined as clinical symptoms of an infection (fever and/or pyuria for urinary tract infection and fever and/or productive cough and radiographic evidence of consolidation for pneumonia) and positive culture data (for both pneumonia and urinary tract infection).¹⁰

Laboratory Studies

Leukocyte counts, plasma cortisol, and ACTH concentrations were determined by the clinical laboratory. Plasma α -MSH concentrations were determined using a commercially available enzyme immunoassay kit (Phoenix Pharmaceuticals, Belmont, CA). Briefly, peptides were eluted from 0.5 mL of acidified plasma using C18-SEP

columns containing 200 mg of C18 (Phoenix Pharmaceuticals, Belmont CA); the samples were evaporated by centrifugal vacuum concentration and reconstituted in 125 μ L of buffered saline before enzyme immunoassay. The concentration of circulating IL-6 was measured with a cytometric bead-based system (Fluorokine MAP, R&D Systems); the lower limit of detection was 1.11 pg/mL. Values below the limit of detection are referred to as not detected and assigned the lowest limit of detection for statistical testing.

Statistics

Descriptive data are presented as median and interquartile range. Group comparisons were performed using the Mann-Whitney U test or Kruskal-Wallis H test. Data were normalized and associations tested using the Pearson correlation. Logistic regression was used to estimate the odds ratio and 95% CI for the effect of the highest initial α -MSH concentration (within 72 hours of stroke onset) on neurological outcome at 1, 3, 6, and 12 months after stroke onset. Given the relatively severe strokes seen in this study, good outcome was defined as independent ambulation (modified Rankin Scale ≤ 3). Significance was set at $P \leq 0.05$.

Results

A total of 114 patients were enrolled in the parent study; plasma α -MSH concentrations were determined in 111 of these patients,

Table 1. Correlations Among α -MSH, ACT, Cortisol, Infarct Volume, and Stroke Severity at 24 Hours and 72 Hours After Stroke Onset

Variable	Infarct Volume*	Uncorrected				Corrected for NIHSS		
		NIHSS	ACTH	Cortisol	IL-6	ACTH	Cortisol	IL-6
α-MSH								
24 h	-0.148, NS	-0.479, $P=0.010$	-0.127, NS	-0.501, $P=0.025$	-0.238, NS	-0.181, NS	-0.256, NS	0.281, NS
72 h	-0.247, $P=0.014$	-0.241, $P=0.016$	-0.139, $P=0.181$	-0.115, NS	-0.209, $P=0.042$	-0.102, NS	0.042, NS	-0.068, NS
Cortisol								
24 h	0.498, $P=0.007$	0.728, $P<0.001$	0.304, $P=0.132$...	0.378, $P=0.100$	0.496, $P=0.014$...	-0.237, NS
72 h	0.420, $P<0.001$	0.616, $P<0.001$	0.346, $P<0.001$...	0.535, $P<0.001$	0.358, $P<0.001$...	0.325, $P=0.002$
ACTH								
24 h	0.069, NS	-0.068, NS	-0.124, NS	-0.118, NS
72 h	0.138, $P=0.161$	0.127, $P=0.193$	0.133, $P=0.197$	0.057, NS
IL-6								
24 h	0.517, $P=0.006$	0.700, $P<0.001$
72 h	0.393, $P<0.001$	0.566, $P<0.001$

Given the significant relationship between stroke severity and α -MSH, cortisol, and IL-6, correlations corrected for stroke severity (using the NIHSS score as a continuous variable) are also presented. Data are normalized and presented as Pearson's r .

MSH indicates melanocyte stimulating hormone; ACTH, adrenocorticotropic hormone; IL, interleukin; NIHSS, National Institutes of Health Stroke Scale; NS, not significant ($P\geq 0.200$).

*Infarct volume is not available for 3 persons.

who are the subject of this article. The characteristics of the overall study population have been described elsewhere.^{9,10} For the 111 patients in whom α -MSH was assessed early (by 72 hours), the median age was 57 years (range: 44–66 years), the median National Institutes of Health Stroke Scale score was 11 (range: 4–19), the median infarct volume was 12 mL (range: 1–80 mL), and 35% of the patients were women. As in other publications related to this study population, we divided patients into tertiles based on stroke severity to assess changes in plasma α -MSH over the course of time.^{9,10} Patients with the most severe strokes (National Institutes of Health Stroke Scale ≥ 17) had lower concentrations of plasma α -MSH than patients with the least severe strokes at both 24 hours and 72 hours after stroke onset (Figure A). There was still a trend toward decreased α -MSH at 1 week after stroke onset in these severely affected patients, but the differences normalized over the course of time. At 1 year after stroke, the median α -MSH concentration among all of the patients was 12.8 pg/mL (range: 6.4–21.1 pg/mL), which is similar to that reported in the literature for healthy adults.¹² Stroke severity appeared to have little impact on plasma ACTH (Figure B), but patients with more severe strokes evidenced increases in cortisol and IL-6 that persisted for ≥ 1 month after stroke onset (Figure C and D).

Early relationships among α -MSH, ACTH, cortisol, and IL-6, as well as the relationships of α -MSH, ACTH, cortisol, and IL-6 with infarct volume and stroke severity, are displayed in Table 1. Table 2 depicts the differences in the highest α -MSH concentration within the first 72 hours after stroke as a function of clinical and demographic differences between patients, none of which are significant after controlling for stroke severity. Initial plasma α -MSH was not predictive of early poststroke infection in either univariate analyses or analyses controlling for covariates (data not shown).

The effect of α -MSH, ACTH, cortisol, and IL-6 on early and long-term outcomes is shown in Table 3. Univariate

associations between initial IL-6 and worse outcomes are seen early after stroke (1 and 3 months), but this effect seems to be related solely to stroke severity. Higher plasma cortisol is independently associated with worse outcomes at 1 month after stroke onset, but this relationship attenuates over the course of time and is lost after controlling for stroke severity and other important predictors of outcome. The effect of early plasma α -MSH concentrations on outcome was not apparent until later time points after stroke and was independent of initial stroke severity, patient age, and infection status.

Discussion

In this study we found early and sustained elevations in both plasma cortisol (to 1 month) and IL-6 (to 6 months) among patients with severe stroke, whereas ACTH concentrations were largely unchanged and α -MSH concentrations decreased early after stroke. That elevated plasma cortisol is seen in patients with severe strokes and is associated with worse outcome is well documented.^{3,5,13–15} Increased cortisol is considered to be a marker of the acute phase/stress response in stroke and is variably attributed to increased ACTH and/or IL-6.^{16,17} We found both plasma cortisol and IL-6 to be highly correlated with stroke severity and infarct volume. As might be expected, there was a correlation between plasma ACTH and cortisol, and this correlation was essentially unchanged after controlling for stroke severity. Also, similar to previous studies, we saw a correlation between IL-6 and plasma cortisol. This correlation was slightly attenuated but not lost after controlling for stroke severity, suggesting that IL-6 may drive some cortisol production independent of stroke severity and ACTH expression. Despite the common origin of α -MSH and ACTH from POMC, the plasma concentrations of these neuropeptides were not correlated after stroke, and the association between plasma α -MSH and stroke severity/infarct volume was not nearly as robust as that seen for cortisol and

Table 2. Differences Between Initial Plasma α -MSH Concentrations (pg/mL) Based on Clinical and Demographic Variables

Patient Characteristics	Variables		Unadjusted <i>P</i>	Adjusted for Stroke Severity <i>P</i>
Sex	Yes	No		
Female	12.2 (6.1–20.5), N=39	11.6 (1.8–17.6), N=72	NS	NS
Ethnicity	Yes	No		
White	11.6 (1.9–17.9), N=100	13.3 (10.5–45.8), N=11	0.140	0.076
Medical history	Yes	No		
AF	16.1 (9.9–27.9), N=16	11.5 (1.9–15.8), N=95	0.080	0.165
CHD	12.3 (4.7–23.5), N=26	11.4 (2.0–17.0), N=85	NS	NS
DM	13.5 (0.6–22.8), N=27	11.6 (4.3–15.5), N=84	NS	0.154
HTN	12.5 (0.5–21.4), N=59	11.0 (6.1–17.1), N=52	NS	NS
Smoker	10.2 (0.6–14.3), N=43	12.6 (4.7–18.7), N=68	0.123	NS
Previous stroke (on imaging)*	13.0 (0.2–22.3), N=26	11.6 (3.7–18.0), N=82	NS	NS
Oxfordshire Stroke Classification	Yes			
TACS (N=17)	7.4 (0.3–11.4)		0.050	0.072
PACS (N=62)	11.8 (3.7–19.3)			
LACS (N=10)	12.5 (6.6–13.7)			
POCS (N=22)	15.4 (6.6–30.2)			
Stroke therapy	Yes	No		
IV tPA	10.5 (3.7–17.3), N=26	11.8 (2.0–18.2), N=85	NS	NS
Endovascular intervention	12.4 (0.5–29.7), N=15	11.8 (2.6–17.4), N=96	NS	0.075
Hemicraniectomy	7.4 (0.2–10.8), N=9	12.3 (4.3–19.3), N=102	0.015	NS
Stroke complications	Yes	No		
Infection within 15 d	10.7 (0.4–13.9), N=26	12.5 (4.3–19.7), N=85	0.112	NS
PNA within 15 d	9.8 (0.3–16.7), N=12	11.8 (4.4–18.4), N=99	0.198	NS

Statistics are by Mann-Whitney *U* test or Kruskal-Wallis *H* test and are either unadjusted or adjusted for stroke severity (using the NIHSS score as a continuous variable).

AF indicates atrial fibrillation; CHD, coronary heart disease; DM, diabetes mellitus; HTN, hypertension; TACS, total anterior circulation stroke; PACS, partial anterior circulation stroke; LACS, lacunar stroke; POCS, posterior circulation stroke; PNA, pneumonia; NIHSS, National Institutes of Health Stroke Scale; NS, not significant; IV tPA, intravenous tissue-type plasminogen activator; MSH, melanocyte stimulating hormone. α -MSH values indicate the highest α -MSH concentration within the first 72 h after stroke onset.

*Three patients did not have MRI imaging and are not included in this analysis ($P \geq 0.200$).

IL-6. Given that the half-life of α -MSH in circulation is on the order of minutes, it is possible that more significant associations between α -MSH and stroke severity were missed because of timing of blood draws.

Despite the limitations of this study with regard to timing of blood draws, we were still able to demonstrate a decrease in plasma α -MSH among patients with severe strokes (National Institutes of Health Stroke Scale ≥ 17) early after stroke onset. To our knowledge, this is the first study that addresses endogenous changes in plasma α -MSH after ischemic stroke, although we did find a similar decrease in plasma α -MSH in an animal study of severe stroke.⁷ Further, we found that higher plasma α -MSH was associated with an increased likelihood of experiencing a good clinical outcome, an effect that was most apparent at later time points after stroke and independent of stroke severity, patient age, and infection status. In contrast, the associations between cortisol and IL-6 on outcome were most robust at early time points

after stroke and explained almost entirely by the fact that cortisol and IL-6 are markers of stroke severity. The lack of an independent association among cortisol, IL-6, and stroke outcome has been documented previously.^{5,18}

Both the independent association of α -MSH with stroke outcome and the delay in this observed association suggest that the effect of early plasma α -MSH on outcome is more than a reflection of the stress response related to stroke severity and that maintenance of plasma α -MSH after stroke onset may be protective. Furthermore, a growing body of experimental data shows that exogenous administration of α -MSH decreases infarct volume and improves stroke outcome.^{7,19–22} There are numerous mechanisms by which α -MSH (and related neuropeptides) could improve stroke outcome, and these effects are mediated through 5 different melanocortin receptors (MCRs). Potent antipyretic properties of α -MSH, which could potentially be capitalized on in the treatment of stroke, are mediated through the MCR3/MCR4

Table 3. Likelihood for a Good Outcome at Given Time Points Based on Initial Plasma α -MSH or Cortisol

Variable	Model	α -MSH		ACTH		Cortisol		IL-6	
		mRS \leq 3	<i>P</i>	mRS \leq 3	<i>P</i>	mRS \leq 3	<i>P</i>	mRS \leq 3	<i>P</i>
1 mo, N=102	Unadjusted	1.355 (0.938–1.957)	0.105	0.852 (0.676–1.075)	0.176	0.169 (0.075–0.381)	<0.001	0.583 (0.389–0.874)	0.009
	NIHSS	1.031 (0.694–1.531)	NS	0.921 (0.688–1.232)	NS	0.394 (0.158–0.986)	0.047	0.896 (0.633–1.269)	NS
	NIHSS+age	1.000 (0.667–1.499)	NS	0.931 (0.698–1.241)	NS	0.389 (0.153–0.988)	0.047	0.915 (0.643–1.302)	NS
	NIHSS+age+infection	0.991 (0.657–1.494)	NS	0.940 (0.693–1.274)	NS	0.392 (0.154–0.998)	0.049	0.940 (0.661–1.337)	NS
3 mo, N=100	Unadjusted	2.147 (1.159–3.978)	0.015	0.840 (0.659–1.070)	0.157	0.299 (0.154–0.581)	<0.001	0.556 (0.375–0.825)	0.004
	NIHSS	1.639 (0.883–3.043)	0.118	0.880 (0.657–1.180)	NS	0.657 (0.307–1.405)	NS	0.755 (0.518–1.102)	0.145
	NIHSS+age	1.568 (0.844–2.911)	0.154	0.896 (0.678–1.183)	NS	0.664 (0.300–1.467)	NS	0.752 (0.503–1.123)	0.163
	NIHSS+age+infection	1.688 (0.859–3.319)	0.129	0.923 (0.682–1.248)	NS	0.721 (0.311–1.669)	NS	0.792 (0.530–1.183)	NS
6 mo, N=97	Unadjusted	5.212 (1.614–16.834)	0.006	0.736 (0.560–0.969)	0.029	0.368 (0.178–0.759)	0.007	0.877 (0.738–1.042)	0.136
	NIHSS	4.219 (1.225–14.530)	0.023	0.741 (0.533–1.032)	0.076	0.724 (0.300–1.747)	NS	1.033 (0.855–1.248)	NS
	NIHSS+age	4.236 (1.191–15.063)	0.026	0.729 (0.534–0.996)	0.047	0.662 (0.254–1.727)	NS	1.030 (0.852–1.246)	NS
	NIHSS+age+infection	5.763 (1.350–24.591)	0.018	0.737 (0.537–1.011)	0.059	0.677 (0.257–1.784)	NS	1.050 (0.863–1.278)	NS
12 mo, N=96	Unadjusted	4.444 (1.322–14.943)	0.016	0.874 (0.657–1.163)	NS	0.464 (0.215–1.000)	0.050	0.873 (0.734–1.039)	0.125
	NIHSS	3.551 (1.034–12.201)	0.044	0.922 (0.683–1.245)	NS	0.937 (0.355–2.473)	NS	1.006 (0.831–1.218)	NS
	NIHSS+age	3.502 (0.991–12.370)	0.052	0.920 (0.625–1.354)	NS	0.859 (0.288–2.563)	NS	0.998 (0.820–1.215)	NS
	NIHSS+age+infection	4.612 (1.087–19.572)	0.038	0.933 (0.630–1.381)	NS	0.933 (0.294–2.955)	NS	1.012 (0.826–1.240)	NS

Data are presented as the odds ratio (95% CI) per 10-pg/mL increase in plasma α -MSH, ACTH, and IL-6 concentrations or 10- μ g/dL increase in cortisol concentration.

Data show the highest plasma α -MSH, ACTH, cortisol, or IL-6 within the first 72 h after stroke.

MSH indicates melanocyte stimulating hormone; ACTH, adrenocorticotropic hormone; IL, interleukin; NIHSS, National Institutes of Health Stroke Scale; NS, not significant ($P \geq 0.200$); CI, confidence interval.

receptor complex in the brain.²³ MCR1 is expressed by cells of the immune system and is responsible for mediating the robust anti-inflammatory and immunomodulatory properties of α -MSH, which include the prevention of T-helper 1 responses and the induction of T regulatory responses to selected antigens.^{24–27} Given the effect of α -MSH on the immune response, it is not surprising that it has been shown to improve outcome in animal models of experimental allergic encephalomyelitis.^{28,29} We also found that α -MSH administration decreased infarct volume and improved neurological outcome 24 hours after transient middle cerebral artery occlusion in an animal model of stroke.⁷ Consistent with the known effects of α -MSH on the immune response, we found that splenocytes harvested from α -MSH-treated animals responded less well to phytohemagglutinin (a lymphocyte mitogen) than splenocytes harvested from saline-treated animals. Furthermore, the animals treated with α -MSH in this study were less likely to develop autoimmune responses to myelin basic protein, a response associated with worse stroke outcome.^{30,31} Finally, α -MSH has neurotrophic properties that could aid in stroke recovery.^{32–37} At least some of these neurotrophic effects appear to be mediated by MCR4.^{36,37} These effects of α -MSH, along with the immunomodulatory effects, may help to explain why delayed administration of α -MSH can improve outcome and why the association between early α -MSH and stroke outcome is not apparent until later time points.^{20,37–39}

α -MSH is an attractive candidate for stroke therapy given its multiplicity of actions and the possibility that delayed administration may still be of therapeutic value. The attractiveness of α -MSH as a therapeutic agent is further enhanced

by its potential ease of administration; MSH-related neuropeptides are absorbed through the nasal mucosa rapidly after inhalation.⁴⁰ In addition to exogenous administration of the neuropeptide, plasma α -MSH concentrations could be augmented by strategies that favor α -MSH processing from POMC/ACTH (ie, enhancing PC2 activity). The potent immunomodulatory properties of α -MSH, however, suggest the possibility that this peptide could predispose to infection, a complication that was seen in an animal model of stroke.⁴¹ In the current study, however, we did not find an independent association between α -MSH and infection risk. Furthermore, we did not see infectious complications related to α -MSH administration in our animal model of stroke.⁷

Limitations of this study include the lack of tightly controlled timing of blood draws early after stroke onset. The median time from stroke onset to the “24-hour” blood draw was 28 hours (N=30), whereas the median time from stroke onset to the “72-hour” blood draw was 68 hours (N=101). It is certainly possible that rapid changes in plasma α -MSH were missed by this sampling protocol. For the logistic regression, we chose to use the highest α -MSH (ACTH, cortisol, and IL-6) in the first 72 hours of stroke onset to increase statistical power (if only the 72-hour values are used, the results are similar but not quite as robust). To better address dynamic changes in α -MSH after stroke, future studies will need to enroll patients as soon as possible after stroke onset and perform frequent assays for both α -MSH and related neuropeptide. Another limitation of this study is the fact that the statistics were not corrected for multiple comparisons; results should, therefore, be interpreted as hypothesis generating.

In summary, decreased plasma α -MSH is seen early after stroke onset in patients with severe stroke. In addition, higher

concentrations of plasma α -MSH are independently associated with better stroke outcome. These data, along with a robust body of experimental data, suggest that strategies to increase α -MSH may be a viable therapeutic intervention for the treatment of acute ischemic stroke and should be further investigated.

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Disclosures

None.

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