The Neuropeptides
Pharmacology Biochemistry and Behavior, Vol. 5, Suppl. 1, pp. 59–64. ANKHO International Inc., 1976.

Effects of α-MSH on Motivation, Vigilance and Brain Respiration

JAAK PANKSEPP, PATRICK REILLY, PAUL BISHOP, RICK B. MEEKER, THOMAS R. VILBERG

Department of Psychology, Bowling Green State University, Bowling Green, OH 43403

AND

ABBA J. KASTIN

Endocrinology Section of the Medical Service, Veterans Administration Hospital

and

Department of Medicine, Tulane University School of Medicine, New Orleans LA 70122

PANKSEPP, J., P. REILLY, P. BISHOP, R. B. MEEKER, T. R. VILBERG AND A. J. KASTIN. *Effects of α-MSH on motivation, vigilance and brain respiration.* PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 59–64, 1976. - In a series of experiments designed to assess the effects of α-MSH on various motivational processes, it was observed that the hormone can slightly decrease food intake and increase water consumption during the first hr after administration in rats. α-MSH also modified avoidance behavior in 1- and 3-day-old chicks, but there were no reliable effects on activity, distress vocalizations and the tonic immobility response. α-MSH appeared to modulate the sleep-waking activity of rats, and the most prominent effect was an increase of slow wave sleep during the 2–3 rd hr after treatment. A possible second effect was a homogenization of sleep patterns – with poor sleepers exhibiting increases of activated sleep and good sleepers a reduction. Measurement of *in vitro* brain oxygen consumption indicated that mice treated with α-MSH exhibit an 18% reduction in respiration of the brain stem section which includes the locus coeruleus, but did not reliably change respiration in forebrain cortices. α-MSH also produced a modest 14% increase of plasma glucose. These results are discussed in terms of possible modulation by α-MSH of activity in central autonomic cell groups such as the locus coeruleus.

α-MSH Motivation Vigilance Sleep Brain stem

UNLIKE the pigmentary effects of melanocyte stimulating hormone (MSH) in amphibians, no clear target organ or function has been found for the substantial titers of this hormone in mammals. Presently, the most likely possibility is that the brain itself is the major target for the action of MSH in higher animals. Certainly a large number of behaviors can be affected by exogenous administration of the hormone. Extinction of both aversive [1] and appetitive tasks [5,22] is increased suggesting that motivational processes which sustain behavioral persistence are intensified. That these effects are not merely due to a decreased capacity to learn new environmental contingencies is indicated by observations that rats learn complex mazes [26] and simple reversal tasks [21] faster after MSH than placebo. Surprisingly, however, if the task requirements [24] or relevant cues [18] are changed, animals tend to exhibit deficits in reversal learning. MSH also has complex emotional effects – increasing dark preference and general emotionality of rats under some conditions [8,27]. Human studies indicate that MSH tends to decrease anxiety and increase visual attention [6, 11, 20].

Still, a definitive function for MSH in mammals has not yet materialized. Although it is conceivable that all the behavioral changes may be produced by a unitary underlying change such as increased attention or certain emotional changes [20], that is yet to be firmly demonstrated. The existing evidence does, however, indicate the need for further investigation of the effects of MSH on basic motivational, emotional and vigilance processes. Accordingly, in the following experiments we investigated the effects of this hormone on a variety of behaviors and related physiological states.

EXPERIMENT 1: FEEDING AND DRINKING

Previous work with appetitive tasks has indicated that hungry rats exhibit delayed extinction when treated with α-MSH [22]. This along with the slight increase of activity exhibited by rats after MSH in certain appetitive situations (7) may indicate that MSH intensifies hunger in rats. Previous work has failed to note any major effects of α-MSH on 2 hr food intake of 22 hr deprived rats, although free water intakes were reliably increased [22]. Similarly, no effect has been observed on daily food intakes of

[1] This work was supported by NSH Grant GB-40150, PHS Grants AM17157 and NS 07664 and a Research Scientist Development Award MH-0086 to the senior author.

unstarved rats during daily administration of MSH [7]. However, if the appetite-modulatory effect of α-MSH is to increase hunger over a brief span of time, effects on food intake could easily have been missed in the above studies. Accordingly, in the following experiments we studied the short- and long-term effects of α-MSH on food and water intake of nondeprived rats.

Method

Twelve mature male Long-Evans rats were housed individually in 14.5 × 7 × 9 cm high wire mesh cages with free access to Wayne powdered laboratory chow from spill retarding containers and water from graduated drinking tubes. Lighting was on a 12 hr light-dark cycle and food and water intakes were recorded at 12 hr or shorter intervals to 0.1 g and 1.0 ml respectively. On the experimental day, all animals were injected intraperitoneally (IP) with 10 μg/rat of α-MSH (10^7 μ/mg) at the start of the dark and light periods. Half the animals received the first injection at the start of each period. To obtain short-term measures of feeding, intakes were recorded hourly for the first 5 hr of the dark cycle.

Results and Discussion

Daily food and water intakes are presented in Table 1. No effect of α-MSH was apparent here or in the diurnal distributions of intakes. The only observation suggestive of a meaningful effect on ingestive behavior occurred during the first hr following MSH administration. During this hour, feeding was reduced 20% from 1.9 to 1.5 g while water intake increased 33% from 3 to 4 ml. Although neither observation alone was statistically reliable, due to the opposing tendencies, the food/water ratio was significantly lower for animals receiving α-MSH ($p<0.05$) with a nonparametric test (Wilcoxon) but not by a parametric comparison (t-test). This small difference had disappeared by the second hour of recording. In general, these data confirm that α-MSH has no major effect on basic ingestive behavior. Certainly there was no increase in hunger. Accordingly previously reported increases in resistance to extinction and increased activity in appetitive tasks cannot be ascribed to such a mechanism. Thus it appears that α-MSH can be used in learning experiments which employ food and water as reinforcers with little confounding by concurrent changes in drive levels.

TABLE 1

DAILY FOOD AND WATER INTAKES DURING 12 HR INJECTIONS OF CARRIER OR MSH

	Pre-day	Treatment Day	Post-Day
Food Intakes (G)			
Carrier	22.1 (±2.2)	20.9 (±2.4)	19.5 (±3.4)
MSH	21.8 (±2.5)	21.1 (±2.1)	19.8 (2.9)
Water Intakes (Ml)			
Carrier	37 (± 4)	39 (±6)	35 (±6)
MSH	36 (±4)	41 (±8)	36 (±9)

Values are means ± SD.

EXPERIMENT 2: EMOTIONAL BEHAVIOR IN YOUNG CHICKS

Unlike most other commonly used laboratory animals, the young chick displays a wide variety of spontaneous and easily measured behaviors in response to isolation in an open field. Because MSH has produced effects which suggest modified emotionality [24], in the following experiment we assessed the effect of α-MSH on activity, distress vocalization, avoidance behavior and tonic immobility of 1- and 3-day-old chicks.

Method

In the first experiment the behavior of 22 one- and three-day-old male Leghorn chicks was recorded in a 90 × 90 cm open-field unit (marked into 9 equal squares) maintained at a constant temperature of 32° C. Activity was measured by the number of squares which the chick entered during the 10 min test session. Distress vocalizations were recorded automatically by microphone and a sound activated relay. Avoidance was measured at the end of the 10 min observation period by the experimenter approaching the bird from the front with an open hand and observing the response. A scale from 1 to 5 was used to rate the avoidance response according to the following criteria: (1) little or no movement away from the hand; (2) slight movement backward from hand (maximum of 2 or 3 steps); (3) continuous walking or backing away from the approaching hand; (4) rapid walking or running from the hand; (5) vigorous undirected running from the hand with wings raised and often accompanied by distress vocalizations. Experimental animals received 4 μg of α-MSH IP 15 min before testing, and controls received equivolumetric doses of the 0.01 M acetic acid :0.9% saline carrier.

In the second experiment, 14 day old chicks were tested for tonic immobility after α-MSH or vehicle. Initially 2 independent groups of 10 animals were tested, and subsequently another 18 birds were tested after both vehicle or MSH (again 4 μg/bird) in a counterbalanced within-animal design, with successive tests being separated by 2 days. Immobility testing was conducted in a room maintained at 24 ± 1° C on a large table partitioned into 4 isolated sections. Induction of the immobility response was accompanied by restraining the animal on its back with the hand for 15 sec followed by a slow, gentle release. The time taken by the bird to right itself was recorded to the closest sec with a stopwatch.

Results and Discussion

In birds tested with the vehicle, mean activity scores were 38 and 4 crosses in 1- and 3-day-old chicks, respectively, indicating the normal development of behavioral inhibition during these ages. Activity of animals tested with α-MSH dropped from 37 to 21 crosses, but the difference at 3 days is not meaningful since it was due exclusively to the extreme agitation of a single bird.

Distress vocalizations were 872 and 340 in 1- and 3-day-old control birds, and these values were not reliably different from the 958 and 312 values of chicks tested with α-MSH.

The average avoidance rating of 1 day old MSH teated animals (2.5 ± 1.4) was reliably higher than exhibited by controls (1.2 + 0.7) (t = 1.83, df = 10, $p<0.05$). At 3 days of age these effects were reversed, animals tested with α-MSH having average scores of 1.2 ± 0.4 and controls 3.0 ±

1.1 (t = 3.38, df = 8, $p<0.01$). The control animals exhibited a clear maturational increase in avoidance which we have typically observed in chicks. The reversal of the effects of α-MSH with age, though outwardly confusing, may reflect previously observed interactions of MSH treatment with level of fear. Since there is a marked development of fear during the first 3 days of life in chicks [15], MSH may facilitate avoidance at low levels of fear and suppress avoidance during high levels of fear. This would be analogous to the interaction of MSH with shock intensity in avoidance situations with rats [25].

The average immobility of birds tested with α-MSH in the experiment with independent groups was 376 ± 306 sec, which was twice as long as the 151 ± 91 sec of controls, but because of the great variability of this behavior the differences were not statistically significant. Similarly, in the within-group experiment, through the trend was in the same direction (458 sec for MSH and 408 sec for control birds), the results were again not reliably different.

EXPERIMENT 3: SLEEP-WAKING PATTERNS

Since one of the major hypotheses concerning action of MSH is that attentional processes are modified [6, 11, 20], a direct analysis of modulation by MSH of vigilance states is indicated. Such a line of inquiry is also suggested by the observation of increased slow wave cortical activity during the first half hr after administration of MSH in rats [19].

Method

Ten male albino rats weighing 311–425 g were surgically prepared with indwelling electrodes for the electroencephalographic measurement of sleep-waking patterns. Cortical activity was monitored via 2 stainless-steel screws threaded unilaterally over the cortex 2 mm anterior and posterior to bregma and 2 mm lateral to the midline. A bipolar electrode was placed into the dorsal hippocampus for the measurement of theta activity. After at least a week of recovery, animals were habituated to sound attenuated sleeping chambers (30 × 30 × 30 in.) and a preliminary sleep record was collected from each animal to verify the scorability of records. Recording was done with Narco Biosystems PMP-4B physiograph at a paper speed of 0.5 cm/sec. Records were visually scored into 3 sleep stages according to traditional criteria. Waking was characterized by low voltage desynchronized activity in cortex and hippocampus; slow wave sleep (SWS) was characterized by a high voltage low frequency activity from both recording sites; activated sleep (AS) was characterized by desynchronized low voltage activity from the cortex and a sustained regular theta activity from hippocampus.

Before each test session, animals were placed in the sleep chambers with food and water at the start of their normal night cycle. Recording was started 12 hr later at the beginning of the light cycle and continued for 4 consecutive hr. Food and water were not available during this period. All animals were tested in a counterbalanced fashion. Vehicle or α-MSH (10 μg/rat) were injected IP immediately at the start of each recording session. Two animals were tested through 3 successive cycles of vehicle and MSH treatment.

Results

Although overall 4 hour values for the vigilance states were not reliably different between the 2 groups, to highlight some possible effects imbedded in these results, hourly data for good and poor sleepers have been analyzed separately (Fig. 1). Poor sleepers were animals which exhibited less than 4% AS (n = 4) and good sleepers, more

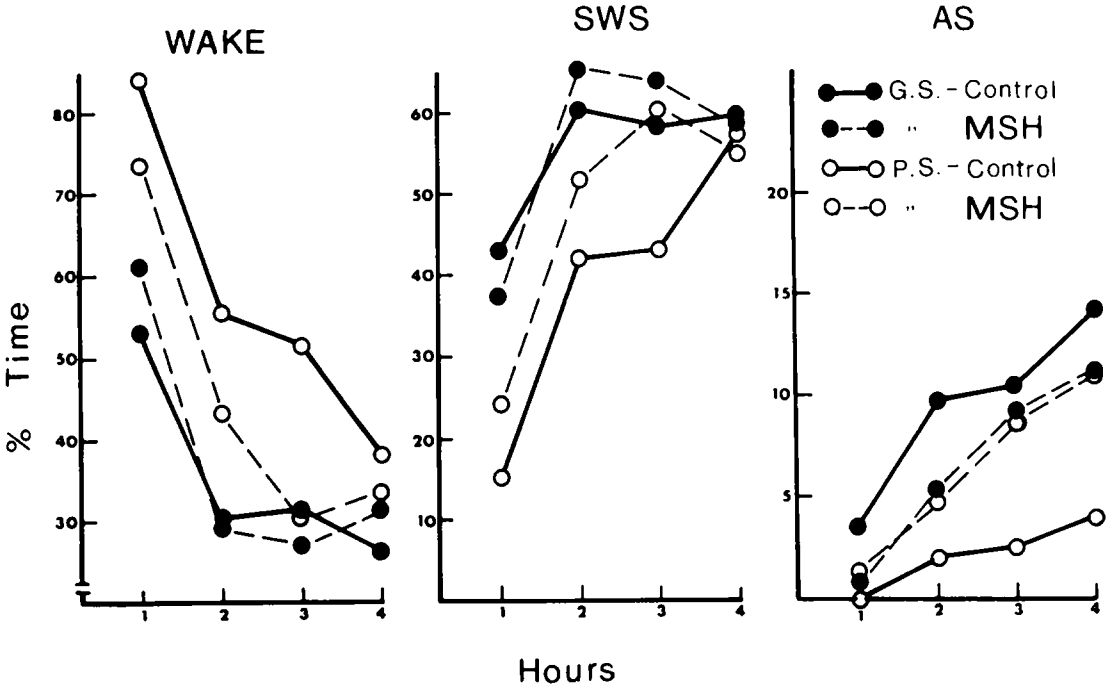

FIG. 1. Hourly sleep waking measures after IP administration of 10 μg/rat of MSH or acidified saline carrier. Animals were divided into good sleepers (G.S.) and poor sleepers (P.S.) according to levels of activated sleep exhibited during the control sessions.

than 6% AS (n = 6) under the vehicle condition. From this analysis, it is apparent that MSH tended to stabilize the sleep pattern of the animals – poor sleepers sleeping more and good sleepers less. This convergence was most apparent for activated sleep – that of good sleepers being reduced by 29% (t = 5.46, df = 5, $p<0.01$) and that of poor sleepers tripled. The increase in AS was due to the doubling of episodes as well as 75% increase in average episode length. In good sleepers, the number of AS episodes was decreased by 20% and episode length by 18%.

The only reliable finding for all animals combined was the 15% increase in slow wave sleep during the second and third hours of recording (t = 2.6, df = 9, $p<0.05$). Again, the poor sleepers exhibited the larger 27% increase while good sleepers exhibited a 9% increment.

The 3 successive recording cycles obtained from 2 animals which had been categorized as poor sleepers indicated that these effects of MSH were not stable across time. In 1 animal the successive changes in AS through the 3 cycles of treatment with α-MSH were first a 260% increase in AS, then a 6% decrease, followed by a 19% decrease. In the other animal, the values were a 490% increase, a 188% increase with a 5% decrease. The observed increase in SWS also diminished systematically with repeated testing. This pattern of results suggests that the effects of α-MSH in poor sleepers was probably due to some aspect of the testing sequence, possibly the initial novelty of the situation.

In general, these results indicate that MSH has relatively little effect on overall sleep-waking activity in rats. Although poor sleepers appeared to rest more and good sleepers less, it should be noted that such a trend could merely be due to statistical regression toward the mean. In any case, the results suggest that this hormone has no profound and consistent effect on the basic mechanisms which control electroencephalographic indices of vigilance. The effects which were observed may be a secondary consequence of a variety of emotional changes that may have been produced by MSH.

EXPERIMENT 4: BRAIN RESPIRATION

MSH has been demonstrated to have several physiological effects on the brain which are compatible with the notion that the hormone might selectively increase the capacity for visual processing that has been observed in human beings [20] and rats [21]. For instance, MSH can increase the power output of occipital cortex [11]. Also, MSH does not reduce blood flow in occipital cortex as in other parts of the brain [3] while at the same time accumulating more in the occipital cortex than other forebrain areas [8]. Because of these findings and the changes in electrocortical activity observed in the previous experiment, we determined whether MSH has any effect on the gross metabolic activity of subregions in the brain as measured by *in vitro* oxygen consumption.

Method

Oxygen consumption was measured by the polarographic technique (Yellow Springs Instrument Co. Model 53 system) which measures oxygen pressure present in a liquid medium. Forty adult male Swiss-Webster mice were used. Half were injected with 4 μg/mouse of α-MSH, and half were injected with equal amounts of the acidified saline medium. Fifteen minutes after injection animals were decapitated, brains were removed, and 4–8 mg samples of occipital cortex and frontal cortex (just dorsal to the rhinal sulcus) were dissected from each brain. Samples of the dorsal brain stem (including the area of the locus coeruleus) (n = 27), diaphragm (n = 15), and liver (n = 20) were obtained from smaller subsets of animals. Each tissue sample was weighed to 0.1 mg and homogenized in 1 ml of freshly aerated mammalian Ringer's solution. The homogenate plus 2 additional ml of aerated Ringer's were incubated at a constant temperature of 37° C and oxygen consumption was monitored for 30 min. At this temperature, the Bunsen coefficient of Ringer's solution is 5 μl of oxygen/ml, yielding a total of 15 μl of oxygen in the 3 ml of medium.

In 13 additional animals, the frontal and/or occipital cortices were sampled before any injections, each sample was divided in half, and 4 μg of MSH or the equivalent amount of vehicle were added to the incubation mediums 30 min after the start of recording. Measurement of respiration was continued for another 30 mins.

Because of the changes in brain respiration observed in the above experiments, plasma glucose levels were determined in 11 α-MSH treated (4 μg/animal) and 10 vehicle treated mice 15 min after injection. Blood was collected after decapitation and glucose levels were measured potentiometrically in triplicate with a Yellow Springs Instruments Glucose Analyzer (YSI Model 23).

Results and Discussion

Tissue 0_2 consumptions after in vivo administration of α-MSH are summarized in Table 2. α-MSH had no reliable effects on tissue respiration except for a significant 18% reduction in oxygen consumption of the dorsal brain stem which included the area locus coeruleus (t = 2.14, df = 35, $p<0.05$). The diaphragm also exhibited a 23% reduction in respiration, but due to the small number of samples and the large variance, the trend was not statistically significant.

TABLE 2

OXYGEN CONSUMPTION (ug/mg/30 MIN) OF BRAIN AND PERIPHERAL TISSUES FOLLOWING IP INJECTIONS OF 4 ug/MOUSE OF MSH OR ACETIC ACID CARRIER 15 MINUTES BEFORE SACRIFICE

Tissue	MSH	Control
Occipital Cortex	0.47 ± .24 (20)	0.46 ± .21 (20)
Frontal Cortex	0.45 ± .17 (20)	0.40 ± .11 (20)
Dorsal Brain Stem	0.23 ± .06* (13)	0.28 ± .05 (14)
Diaphragm	0.36 ± .25 (7)	0.47 ± .36 (8)
Liver	0.23 ± .06 (10)	0.23 ± .04 (10)

Values are Means ± SD.
Numbers in parentheses indicate number of observations.
*$p<0.05$.

Administration of α-MSH into the respiring medim of frontal and occipital tissues had no effect on respiration. For occipital cortex, the control value was 0.34 (± 0.20) and the α-MSH one 0.33 (± 0.20) μg 0_2/mg wet tissue/30 min. The corresponding values for frontal cortex were 0.23 (± 0.05) and 0.26 (± 0.06) μg 0_2/mg wet tissue/30 min.

Essentially these results indicate that α-MSH has very little effect on energy metabolism of the brain. The

reduction of dorsal brain stem respiration was probably secondary to changes in blood flow because it was noted during tissue dissection that this region as well as the brain generally was markedly blanched in animals treated with α-MSH. This probably corresponds to the reduced blood flow that has been reported for many regions of the brain following α-MSH treatment [3]. Of course, if this were the only physiological change that resulted from the α-MSH, other parts of the brain should have also exhibited some reduction in respiration. In fact, the frontal cortex exhibited a 12% (though not significant) increase in oxygen consumption after treatment with α-MSH. Since this heightened respiration in the presence of decreased blood flow might have occurred if increased substrates had been made available to the brain we decided to measure plasma glucose levels after α-MSH. The results indicated that glucose levels were 14% higher in animals treated with α-MSH than in controls (143 mg% vs 126 mg%) ($t = 1.9$, $df = 19$, $p<0.05$). Since in previous work it has been demonstrated that exogenous administration of glucose can increase the respiratory activity of brain tissue [14], it is suggested that reduced forebrain blood flow may have been counteracted by increased endogenous metabolic substrate availability.

GENERAL DISCUSSION

The present results do not lead to an unambiguous unitary hypothesis about the role of α-MSH in the functioning of higher animals. All of the positive effects observed in the present experiments were weak and in some instances ephemeral. However, 4 positive observations were made, and they may be of some importance in delineating the normal biological effect of MSH in nonampibians.

First, the avoidance behavior of young chicks to an approaching object was increased at 1 day of age and decreased at 3 days of age. This finding supports the considerable evidence linking α-MSH to the modulation of fear motivated behaviors [1,9], and further suggests that the type of effect one observes may be dependent on the level of fear. Since emotional behaviors of the young chick are developing rapidly during the first few days of life, the reversal of the effect of α-MSH may not be as problematic as it may initially seem. If the vigor of avoidance in the chick is an inverted U shaped function with increasing age, then MSH might shift the animal up on the curve at a young age and down at an older age. This result would also be expected if α-MSH interacts with fear-motivation in such a way that it improves behaviors induced by low levels of fear and reduces those provoked by high levels. Indeed, this kind of interaction has been observed in rats when intensity of shock is manipulated at low shock intensities MSH facilitates avoidance and at high levels avoidance is reduced [25].

Second, α-MSH appears to have subtle effects on vigilance as measured by electroencephalographic indices of sleep-waking activity. Slow wave sleep is increased during the second and third hr after hormone administration. This observation is compatible with early reports that some rats tested with β-MSH exhibited behavioral somnolence [17] and also possibly with the high incidence of yawning and stretching observed in dogs after MSH administration [2]. This finding may also be compatible with recent observations that α-MSH increases the turnover of brain norepinephrine during the first hr after administration followed by a decreased turnover for several hours [10]. If norepinephrine subserves cortical arousal [4,12], a decrease in norepinephrine turnover may have promoted the appearance of slow wave sleep. If this were the case, we should also have observed a period of increased arousal during the beginning of testing. However, we noted no such effect. The average latency to slow wave sleep in control animals was 30.3 min and in animals treated with α-MSH it was 25.7 min. In fact, 8 of 10 rats studied entered slow wave sleep faster after MSH than after vehicle. Conversely, 8 of the 10 animals took longer to enter activated sleep after α-MSH than vehicle. It might be noted that some animals did tend to exhibit initial sleep patterns which may have been characterized by atypically rapid cycling between slow wave sleep and waking, as if sleep were being disrupted by an arousal process, but this observation was not at all consistent across animals. In fact, half the animals exhibited larger average episode lengths of slow wave sleep than controls. Another possible effect of α-MSH was a reduction in the variability of sleep stages across animals, and this effect was most prominent for activated sleep. This suggests that α-MSH may have a stabilizing effect on a brain mechanism which controls electrocortical arousal. This homogenization of electrocortical activity may also be noteworthy from the perspective that α-MSH has previously been noted to reduce the variability of other behaviors, for instance, the number of errors rats make in learning a complex maze [26]. Certainly it is possible that treatment with α-MSH stabilizes a physiological substrate which normally exhibits either substantial individual or temporal variability.

Our third major observation was that MSH decreased *in vitro* respiration of the dorsal brain stem. This effect was probably due to decreased blood flow to that region, not only because the animals treated with α-MSH exhibited a visually obvious blanching of brain tissue, but also because MSH has been demonstrated to reduce blood flow to many regions of the brain [3]. The fact that respiration was not decreased and may, in fact, have been increased in forebrain areas such as frontal and occipital cortices, suggested that any metabolic effects of reduced blood flow was being homeostatically counteracted by an increase in energy availability.

This idea provoked our fourth observation, namely that α-MSH produced a reliable though modest increase in blood glucose levels of nonfasted mice. Whether this effect was due to a direct action on the liver as opposed to some secondary hormonal change such as increased secretion of epinephrine, glucagon, or glucocorticoids or decreased insulin, cannot be ascertained from the present data. Certainly, from the perspective that MSH is recruited in response to both physical and psychological stressors [23], a blood glucose increase can be seen to be an adaptive response in preparing the brain and body to cope with a stressful situation. Still, it should be emphasized that the hyperglycemic effect of α-MSH was very small, but it may explain why we observed a slight reduction in feeding and an increase of drinking in our first experiment. The increased availability of metabolic substrates may have depressed appetite while any increase in blood tonicity may have induced the animal to drink a little more. Also, it is conceivable that the metabolic changes we observed after α-MSH may have participated in some of the modification in sleep waking patterns since it is clear that changes in

glucose metabolism can modify vigilance states quite markedly [13].

Although the present results do not provide a conclusive mechanism for MSH action, it should be noted that the results are consistent with the possibility that MSH may have as target organ certain pigmented cells of the central autonomic nervous system. For instance, it has recently been demonstrated that the locus coeruleus controls cerebral bloodflow [16], and it was in the brain sample containing this area that a reliable reduction of tissue respiration was observed. Further, since this part of the brain is known to participate in organizing stress related responses and states of vigilance [4], the changes in sleep-waking activity and emotional behaviors in chicks could have been mediated by changes in the activity of pigmented brain stem cell groups such as the locus coeruleus.

Although speculative at this stage, the hypothesis that MSH may modulate central autonomic tone could provide a unifying principle which might account for the many effects of MSH that have been described in the literature and the wide variety of effects which were observed in the present series of experiments. If MSH does modify autonomic tone in the nervous system, it would be expected that a large variety of behaviors would be affected, but not necessarily to any great extent, and the behavioral literature does indicate that the effects of MSH are widespread though often quite weak and sensitive to slight environmental and task changes. Also, from such an autonomic action, it would be reasonable that the behavior of animals in many situations would be stabilized rather than shifted profoundly in any unitary direction, and again it has been occassionally noted that MSH tends to reduce the variability in behavior. Further, since recent work suggests that an increased attention hypothesis of MSH action may be the best general principle available for organizing the diverse behavioral effects of the hormone [11,20], a reasonable mode of action for such a process could be via brain stem cell groups such as the locus coeruleus which have been implicated in the basic maintenance of cortical arousal. Finally, a most compelling fact which is leading us to analyze the role of such brain areas in MSH action is their prominent pigmentation and hence their outward resemblance to the epidermal target tissue of amphibians.

REFERENCES

1. de Wied, D. Effects of peptide hormones in behavior. In: *Frontiers in Neuroendocrinology*. New York: Oxford University Press, 97–140, 1969.
2. Ferrari, W., G. L. Gessa and L. Vargiu. Stretching activity in dogs intracisternally injected with synthetic melanocyte-stimulating hexapeptide. *Experientia* **17**: 90, 1961.
3. Goldman, H., C. A. Sandman, A. J. Kastin and S. Murphy. MSH affects regional perfusion of the brain. *Pharmac. Biochem. Behav.* **3**: 661–664, 1975.
4. Jouvet, M. The role of monomaines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. *Ergeb. Physiol.* **64**: 165–307, 1972.
5. Kastin, A. J., G. L. Dempsey, B. LeBlanc, K. Dyster-Aas and A. V. Schally. Extinction of an appetitive operant response after administration of MSH. *Horm. Behav.* **5**: 135–139, 1974.
6. Kastin, A. J., L. H. Miller, D. Conzalez-Barcena, W. D. Hawley, K. Dyster-Aas, A. V. Schally, M. L. V. DeParra and M. Velasco. Psycho-physiologic correlates of MSH activity in man. *Physiol. Behav.* **7**: 893–896, 1971.
7. Kastin, A. J., M. C. Miller, L. Ferrell and A. V. Schally. General activity in intact and hypophysectomized rats after administration of melanocyte-stimulating hormone (MSH), melantonin, and Pro-Leu-Gly, NH_2. *Physiol. Behav.* **10**: 339–401, 1973.
8. Kastin, A. J., C. Nissen, K. Nikolics, K. Medzihradszky, D. H. Coy, I. Teplan and A. V. Schally. Distribution of ^{3}H-α-MSH in rat brain. *Brain Res. Bull.* **1**: 19–26, 1976.
9. Kastin, A. J., G. A. Sandman, L. O. Stration, A. V. Schally and L. H. Miller. Behavioral and Electroencephalagraphic changes in rats and man after MSH. In: *Progress in Brain Research, Vol. 42*, Amsterdam: Elsevier, 1975, pp. 143–150.
10. Kostrzewa, R. M., A. J. Kastin and Ml S. Spirtes. α-MSH and MIF-I effects of catecholamine levels and synthesis in various rat brain areas. *Pharmac. Biochem. Behav.* **3**: 1017–1023, 1975.
11. Miller, L. H., A. J. Kastin, C. A. Sandman, M. Fink and W. J. Van Veen. Polypeptide influences on attention, memory and anxiety in man. *Pharmac. Biochem. Behav.* **2**: 663–668, 1974.
12. Panksepp, J., J. F. Jalowiec, P. J. Morgane, A. J. Zolovick and W. C. Stern. Noradrenergic pathways and sleep-waking states in cats. *Expl Neurol.* **41**: 233–245, 1973.
13. Panksepp, J., J. F. Jalowiec, A. J. Zolovick, W. C. Stern, and P. J. Morgane. Inhibition of glycolytic metabolism and sleep-waking states in cats. *Pharmac. Biochem. Behav.* **1**: 117–119, 1973.
14. Panksepp, J. and P. Reilly. Median and lateral hypothalamic oxygen consumption as a function of age, starvation and glucose administration in rats. *Brain Res.* **94**: 133–140, 1975.
15. Phillips, R. F. and P. B. Siegel. Development of fear in chicks of two closely related genetic lines. *Animal Behav.* **14**: 84–88, 1966.
16. Raichle, M. E., B. K. Hartman, J. O. Eichling and L. G. Sharpe. Central noradrenergic regulation of cerebral blood flow and vascular permeability. *Proc. natn. Acad. Sci.* **72**: 3726–3730, 1975.
17. Sakamoti, A. Hypersensitivity induced in albino mice by melanocyte-stimulating hormone. *Nature* **211**: 1370–1371, 1966.
18. Sandman, C. A., W. Beckwith, M. M. Giddis and A. J. Kastin. Melanocyte-stimulating hormone (MSH) and overtraining effects on extradimensional shift (EDS) learning. *Physiol. Behav.* **13**: 163–166, 1974.
19. Sandman, C. A., P. M. Denman, L. H. Miller, J. R. Knott, A. V. Schally and A. J. Kastin. Electroencephalographic measures of melanocyte-stimulating hormone activity. *J. comp. physiol. Psychol.* **76**: 103–109, 1971.
20. Sandman, C. A., J. M. George, J. D. Nolan, H. Van Riezen and A. J. Kastin. Enhancement of attention in man with ACTH/MSH 4–10. *Physiol. Behav.* **15**: 427–431, 1975.
21. Sandman, C. A., A. J. Kastin and W. D. Alexander. Neuroendocrine influences on visual discrimination and reversal learning in the albino and hooded rat. *Physiol. Behav.* **11**: 613–617, 1973.
22. Sandman, C. A., A. J. Kastin and A. V. Schally. Melanocyte-stimulating hormone and learned appetitive behavior. *Experientia* **25**: 1001–1002, 1969.
23. Sandman, C. A., A. J. Kastin, A. V. Schally, J. W. Kendall and L. H. Miller. Neuroendocrine responses to physical and psychological stress. *J. comp. physiol. Psychol.* **84**: 386–390, 1973.
24. Stratton, L. O. and A. J. Kastin. Melanocyte-stimulating hormone in learning and extinction of two problems. *Physiol. Behav.* **10**: 689–692, 1973.
25. Stratton, L. O. and A. J. Kastin. Avoidance learning at two levels of shock in rats receiving MSH. *Hormones Behav.* **5**: 149–155, 1974.
26. Stratton, L. O. and A. J. Kastin. Increased acquisition of a complex appetitive task after MSH and MIF. *Pharmac. Biochem. Behav.* **3**: 901–904, 1975.
27. Stratton, L. O., A. J. Kastin and W. P. Coleman. Activity and dark-preference responses of albino and hooded rats receiving MSH. *Physiol. Behav.* **11**: 907–909, 1973.