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Disappearance and Excretion of Labeled α-MSH in Man[1]

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REDDING, T. W., A. J. KASTIN, K. NIKOLICS, A. V. SCHALLY AND D. H. COY. *Disappearance and excretion of labeled α-MSH in man*. PHARMAC. BIOCHEM. BEHAV. 9(2) 207–212, 1978.—Despite the considerable evidence for the CNS actions of α-melanocyte-stimulating hormone (α-MSH) in man, little attention has been given to its half-time disappearance from plasma and urinary excretion in normal individuals. In the first experiment, a healthy man was given 15 μCi of tritiated (^{3}H)-α-MSH as a rapid IV injection. A plot of the disappearance time in plasma was characteristic of a multiexponential curve, the linear components of which were resolved by the subtraction method and half-time disappearance calculated directly from the slope of the regression line. The half-time disappearance was 1 min for the first component and 25 min for the second component. After the IV administration of 50 μCi of ^{125}I-α-MSH in the second experiment, the two components showed halftime disappearances of 1 min and 4.8 min respectively. These times were not changed by precipitation of the plasma with 10% trichloroacetic acid. Thirty-eight percent and 42% of the label appeared in the urine 4 hr after the injection of either ^{3}H-α-MSH or ^{125}I-α-MSH. The results suggest that the persistence of high levels of α-MSH in the blood after injection in man may be too short to fully explain the CNS effects of α-MSH.

α-MSH Peptide Hormone Half-life

THE ECOLOGICAL significance of the role of MSH in amphibians and reptiles appears mainly as an adaptation in color to avoid their predators, but its phylogenetic persistence and physiological role in higher mammals are less well understood. The first study of the extrapigmentary effects of MSH in man was published 10 years ago [10]. Since that time, a number of clinical investigations with α-MSH as well as its active peptide core (MSH 4-10) have been reported [11]. Studies of the distribution of immunoreactive α-MSH or radioactivity after the injection of ^{125}I-labeled-α-MSH or tritiated α-MSH in rats have shown high concentrations in the pituitary and pineal glands as well as in certain regions of the brain [3, 14, 20, 21]. Labeled α-MSH has been used to study its half-time disappearance in rats [4,14], but this method has not been used in man. Accordingly, we attempted to obtain a better understanding of how α-MSH is metabolized and excreted in the human being.

METHOD

Iodinated α-MSH was prepared by the chloramine T method described by Hunter *et al.* [6]. This material was then purified on QUSO by extraction in 0.1 N HCl. Purification was verified by chromatoelectrophoresis as reported elsewhere [14]. This material along with 1 mg of unlabeled α-MSH was passed through a millipore filter and lyophilized to dryness under sterile conditions. Iodination, purification, and sterilization were performed not more than 24 to 48 hr before the experiment in order to minimize the formation of breakdown products. An hour before the beginning of the experiment, the radioactive α-MSH was dissolved in 5% dextrose in water. A healthy man, weighing 76 kg and without any known endocrine disorders, was treated several hours before injection with five drops of Lugol's solution to block thyroidal uptake of the iodinated material. Undamaged ^{125}I-α-MSH (50 μCi) was injected in the volume of 1 ml as an IV bolus in one arm and heparinized blood samples were withdrawn from the opposite arm at 1–2 min intervals during the first 20 min of the experiments and then at 10 to 60 min intervals. Plasma was separated by centrifugation and aliquots counted for radioactivity in a well-type gamma [scintillation] counter (Packard).

Tritiation was performed by catalytic hydrogenation of the 3,5 dibromo-L-tyrosine-2 derivative of α-MSH which had been prepared by classical procedures of peptide synthesis [19]. This material was further purified and characterized by chromatography on carboxymethyl cellulose and thin layer chromatography as previously described [14]. After the addition of 1 mg of unlabeled α-MSH, the material was handled as described above for the iodinated α-MSH

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TABLE 1

PREPARATION AND BIOLOGICAL ACTIVITY OF LABELED α-MSH

Substance	Method of Preparation	Method of Purification	Biological Activity	Specific Activity
^{125}I-α-MSH	chloramine T	extraction with QUSO confirmed by chromatoelectrophoresis	0.3×10^5 U/mg	100 mCi/mg
^{3}H-α-MSH	catalytic tritiation of 3,5 dibromo-L-tyrosine-2 derivative of α-MSH	elution from carboxy-methyl-cellulose with 0.2 M ammonium acetate buffer pH 7.0	1×10^6 U/mg	0.5 mCi/mg

except that plasma and urine samples were counted in a liquid scintillation spectrometer after digestion with 1 ml NCS reagent (Amersham/Searle) and addition of scintillation-fluid. Some of these plasma samples were precipitated with equal volumes of 10% trichloracetic acid and centrifuged; the precipitate was then counted. The experiment using the tritiated MSH was completed several weeks before the injection of ^{125}I-α-MSH in order to avoid high background counts which would have occurred if ^{125}I had been injected first.

The disappearance times were plotted on semilogarithmic coordinates and were characteristic of multiexponential curves. The separate exponential components of the resulting curves were subtracted as straight lines by the standard method of Robertson [22]. The slopes of these lines were calculated by the method of least squares and the half-time disappearance measured directly from the slope. The distribution volume was calculated by extrapolation of the disappearance line to zero time to obtain cpm/ml (^{125}I-α-MSH) or dpm/ml (^{3}H-α-MSH) plasma which was then divided into the injected radioactivity.

Urine samples were collected at 30 to 60 min intervals for 4 hr and also overnight. Aliquots were taken for counting and urine from the experiment involving ^{3}H-α-MSH was saved for further processing. Urine collected during the first 4 hr after injection of the ^{3}H-α-MSH was pooled and lyophilized to dryness. This material was extracted with acidified methanol and evaporated to dryness under reduced pressure; it was re-extracted with acetone and dried. The acetone powder was dissolved in 0.02 M ammonium acetate buffer, pH 4.5, and an aliquot applied to a 1×40 cm carboxymethyl cellulose column previously equilibrated with the same buffer. The column was eluted with 0.02 M ammonium acetate buffer, pH 4.5, at a rate of 1 ml/5 min. After 10 tubes had been collected, the buffer was changed to 0.2 M ammonium acetate, pH 7.0, and elution continued. Aliquots were taken for the determination of radioactivity, pH, and peptide content (ultraviolet absorption at 280 nm). Peak radioactive areas were lyophilized to dryness and chromatographed on thin layer silica gel plates (250 μ).

RESULTS

Table 1 shows the method of preparation, purification, and biological activity of the two preparations of radioactive α-MSH used in our studies. Both the iodinated α-MSH and the tritiated α-MSH were determined to be biologically active by an in vitro assay involving darkening of frog skin [13].

A plot of the disappearance of radioactivity in plasma obtained after injection of the ^{125}I-α-MSH was characteristic of a multiexponential curve (Fig. 1). Within 5 min, over 50% of the radioactivity had disappeared from the blood. The curve appeared linear between 60–240 min and the best line was fitted by inspection through the terminal portion of the curve and extrapolated back to zero time. The half-time disappearance of this exponential curve was about 180 min. When the extrapolated curve was subtracted from the original curve, point by point in their areas of overlap, a second linear curve with a half-time disappearance of 4.8 min was resolved. Extrapolation of this first subtraction curve to the zero intercept on the ordinate gave a second straight line component. By subtraction of this component from the curvilinear part of the exponential curve in their areas of overlap, a second straight line was obtained with a half-time disappearance of 1 min.

The distribution volume of the iodinated α-MSH expressed as percent body weight was 4–5% or about that of the plasma volume. Calculation of the distribution volume from the first subtraction curve (4.8 min) indicated that within this time iodinated α-MSH and its metabolites had been diluted into a pool somewhat greater than the estimated extracellular fluid space.

When the plasma obtained after injection of ^{125}I-α-MSH was precipitated with 10% trichloracetic acid and the precipitate counted, similar exponential curves resulted. A disappearance time of 4 min was found for the first extrapolated component of the exponential curve and 1 min for the second extrapolated component.

Figure 2 shows the disappearance of radioactivity from plasma after the IV injection of 15 μCi of ^{3}H-α-MSH into the same volunteer. When plotted semilogarithmically, the disappearance curve appeared linear from 90 min to 240 min. Over 90% of the radioactivity disappeared within the first 5 min. When the extrapolated curve was subtracted from the original curve, point by point, a linear component was resolved with a half-time disappearance of 25 min. By subtracting this linear component from the initial curvilinear portion of the curve, another straight line with a half-time disappearance of 1 min was resolved. Calculation of the distribution volume from this second subtraction curve indicated that ^{3}H-α-MSH was initially distributed in a pool smaller than that of the estimated plasma volume.

Figure 3 shows the percent urinary excretion of both the

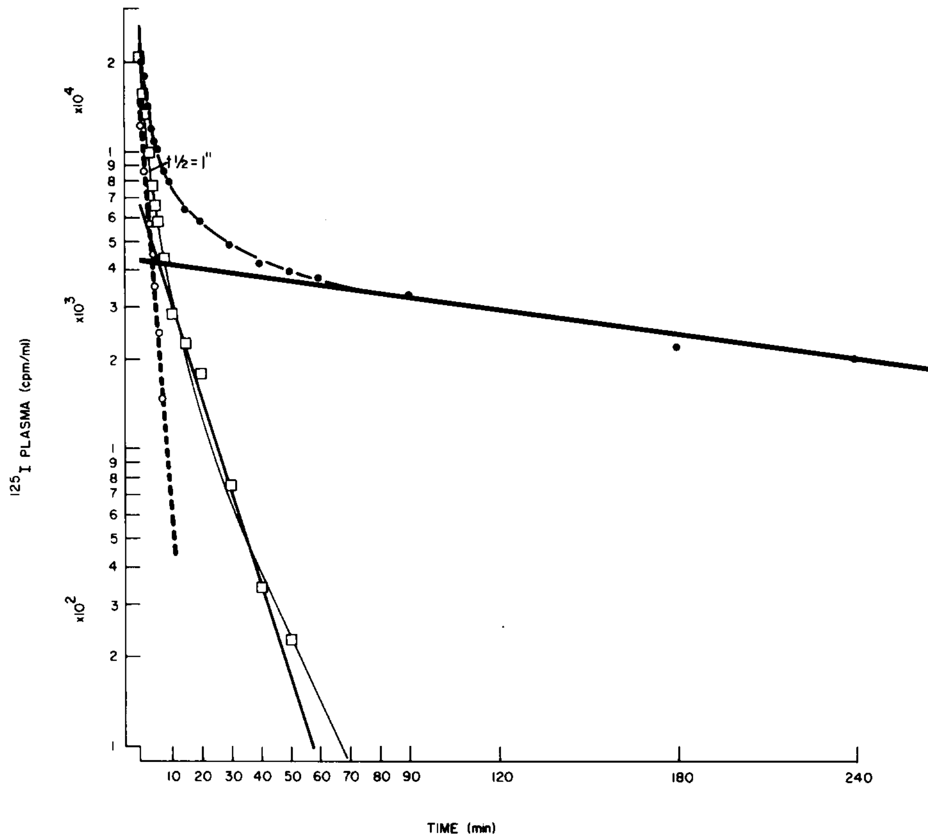

FIG. 1. Disappearance of radioactivity from human plasma after the intravenous injection of 50 μCi of ^{125}I-α-MSH. When the calculated regression points are subtracted from the original values, the resultant linear components are resolved with half times of 1 min and 4.8 min.

tritiated α-MSH and the iodinated α-MSH. In four hours, about 38% of the tritiated dose and 42% of the iodinated dose of α-MSH appeared in the urine while at 24 hr 52.5 to 82% of the tritiated and iodinated doses, respectively, had been excreted into the urine.

An attempt was made to isolate and identify the radioactivity in the urine collected during the first 4 hr after the injection of the tritiated α-MSH. Figure 4 shows the profile of elution of radioactivity from the column. Over 70% of the radioactivity appeared early in the elution (tubes number 9–15); its location suggested an acidic peptide (peak I). Three other small peaks appeared between tubes 21 through 39 and another small peak representing less than 3% of the total radioactivity was found in tubes 76 through 81. Thin layer chromatography of the peaks on silica gel plates along with known standards failed to identify these metabolites although there was some suggestion in preliminary determinations based only on Rf and color reaction to chlorine-0-tolidine spray that the major peak might be similar to that of 1-13-OH-α-MSH.

DISCUSSION

It is tempting to speculate that during phylogenetic evolution MSH has lost the physiologic role it plays in skin pigmentation and has gradually acquired some new functions in mammals. By analogy, oxytocin is a pituitary hormone that appeared in nonmammalian vertebrates in which milk ejection does not exist.

The form in which MSH exists in the human body is not certain, but current evidence suggests it circulates as lipotropin and not as α-MSH. Nevertheless, MSH activity exists in man and occasionally its release may be dissociated from that of ACTH [2, 5, 7, 9, 15, 17, 23]. In addition, α-MSH affects the human brain [1, 10, 12].

The activity and duration of action of a hormone, such as α-MSH, is affected by its dilution, plasma disappearance rate, distribution, receptor binding, metabolism and the urinary clearance of these metabolites from the body. The plasma disappearance time for the fast component of the exponential curve reported here for man as 1 min agrees with the studies reported for the rat in vivo [4,14] and in vitro [16]. This 1 min half-time disappearance for the first component probably represents the transit time required for dispersal of the peptide throughout the capillary beds and rest of the general circulation. Although it is unlikely that there is instantaneous mixing of any peptide, the half-time disappearance of peptides calculated from the initial fast component of the exponential curve will be similarly rapid. This consideration should influence the evaluation of such calculations as half-life, zero time, and distribution volume.

The second exponential curve is more likely to represent the biological half-life of α-MSH in man; this was found to be 4.8 min for the iodinated α-MSH and 25 min for the tritiated α-MSH. The differences in the results obtained with the two

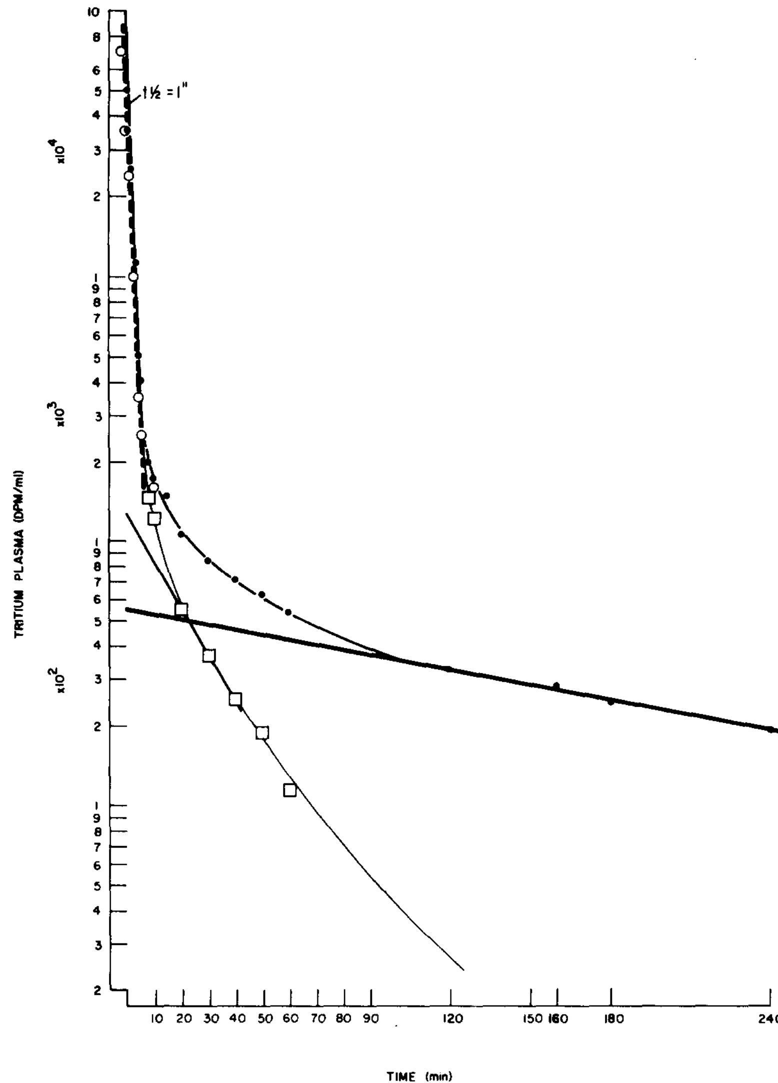

FIG. 2. Disappearance of radioactivity from human plasma after the intravenous injection of 15 μCi of ^{3}H-α-MSH. When the calculated regression points are subtracted from the original values, the resultant linear components are resolved with half times of 1 min and 25 min.

materials can be explained by the differences in labels and labeling methods. Iodination of α-MSH results in a molecule much larger than the natural peptide with concomitant changes in its molecular shape and size which alter its spatial configuration and interactions with enzymes or receptors. The tritiated α-MSH, however, more closely approximates unlabeled α-MSH since the van der Waals radius of tritium is very close to that of hydrogen. The half-time disappearance of 25 min determined from the second component of the exponential curve for the tritiated α-MSH is similar to the value of 20.8 min found by Ashton *et al.* [1] who infused α-MSH for 10 min and then used a specific radioimmunological assay to measure the disappearance of α-MSH. It cannot be assumed by either method that the peptide being measured remains intact after injection. Enzymatic degradation can result in measurement of only the portion of α-MSH containing the radioactive label or the immunologically active amino acid sequence rather than biologically active material; however, there may be a slower rate of breakdown of α-MSH in human blood than in rat blood [16]. Large differences in half-life values determined by these two methods were found for ACTH, also [18]. It is somewhat paradoxical that α-MSH, with a short half-life in the body, can exert CNS effects on behavior and EEG changes which persist for hours [8,14]. This dichotomy may best be explained by its quick initiation of secondary events.

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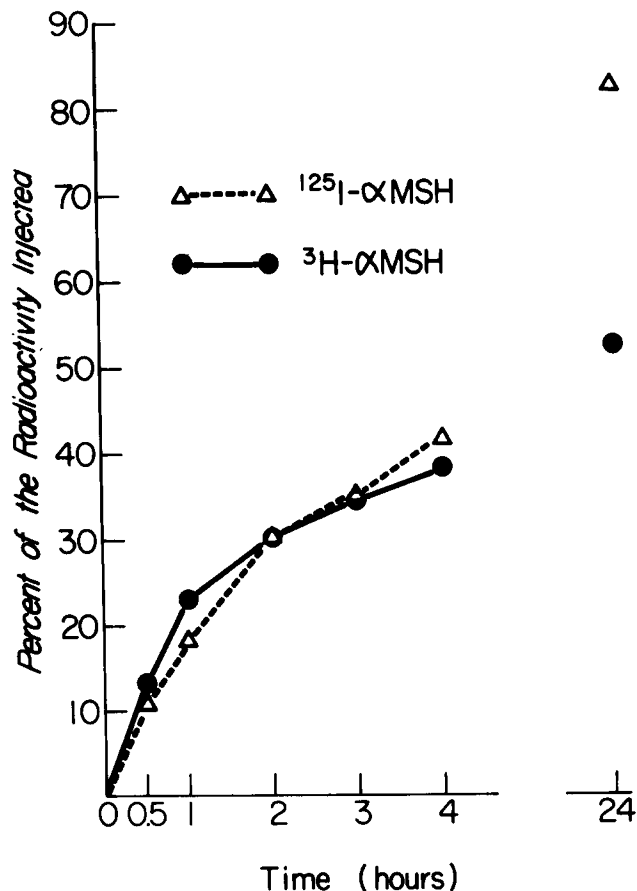

FIG. 3. Percent accumulative urinary excretion of radioactivity after the IV administration of ^{3}H-α-MSH or ^{125}I-α-MSH.

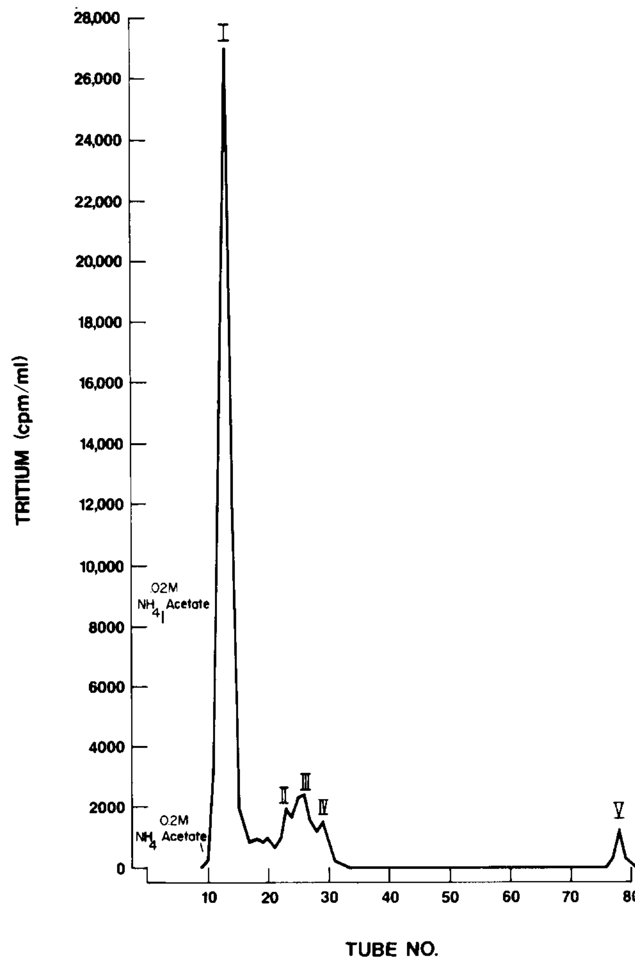

FIG. 4. Chromatography of methanolic-acetone extract of urine on carboxymethyl cellulose. The radioactivity was eluted with 0.02 M and 0.2 M ammonium acetate buffers, pH 4.5 and 7.0 respectively, as indicated by short lines. Peak areas of radioactivity are shown with Roman numerals and were subsequently rechromatographed by thin layer chromatography on silica gel.

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