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Peptides Readily Penetrate the Blood-Brain Barrier: Uptake of Peptides by Synaptosomes is Passive[1,2]

R. GREENBERG, C. E. WHALLEY, F. JOURDIKIAN[3]
I. S. MENDELSON AND R. WALTER

Department of Physiology, University of Illinois Medical School Chicago, Illinois 60680 and [3]Department of Research, Natl. College of Chiropractics, Lombard, Illinois 60148

AND

K. NIKOLICS, D. H. COY, A. V. SCHALLY AND A. J. KASTIN

Endocrinology Section of the Medical Service and Endocrine and Polypeptide Laboratory, Veterans Administration Hospital, Department of Medicine, Tulane University School of Medicine, New Orleans LA 70140

GREENBERG, R., C. E. WHALLEY, F. JOURDIKIAN, I. S. MENDELSON, R. WALTER, K. NIKOLICS, D. H. COY, A. V. SCHALLY, AND A. J. KASTIN. *Peptides readily penetrate the blood-brain barrier: uptake of peptides by synaptosomes is passive.* PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 151–158, 1976. – The intracarotid artery quick injection technique of Oldendorf was utilized to determine the Brain Uptake Index (BUI) of radio-labeled peptides in comparison with ^3H_2O or ^{14}C-antipyrine as counterlabels. The normalized BUI values for ^3H-MIF-I, ^3H-α-MSH and ^{14}C-AVP were 13.7, 9.6 and 13.0, respectively at 15 sec after injection consistent with their having readily penetrated the blood-brain barrier. The BUI values were similar, though somewhat increased, at 10 min postinjection consistent with their ready exit across the blood-brain barrier. At 15 sec after injection 0.5 ± 0.1%/g brain of the originally injected peptide label was recovered; and 0.1 ± 0.2%/g brain was recovered after 10 min. The label was distributed uniformly in the major brain regions at both times. However, the percentage of the originally injected label/g of pineal and pituitary gland tissue was 10–20 × increased as compared with the major brain regions as would be expected by their location outside the blood-brain barrier. The in vitro uptake of the radio-labeled peptides by synaptosomes prepared from the whole brain and the major brain regions was passive; it was not temperature dependent, nor was it Na^+ dependent. However, the binding of the three peptides by the synaptosomes varied considerably: AVP>MSH>MIF: 50>5>1. The penetration of the blood-brain barrier by the three peptides is consistent with their having CNS effects.

Peptides α-MSH MIF-I AVP Blood-brain barrier Brain uptake index Synaptosome uptake Peptide binding Passive uptake

THERE are increasing circumstantial data to suggest that peptides exert pharmacologic effects within the CNS after parenteral administration [3,12]. Behavioral changes have been observed in animals and in man [16, 22, 41], presumably as a consequence of the penetration of the injected peptides through the choroid-CSF barrier and/or the blood-brain barrier [29,30] after which they may modify neural electrical activity [2, 12, 24, 34] or be sequestered either for later release or for intracellular degradation. Since several of the endogenous peptides are highly localized to nerve endings [20, 27, 28, 39], and since several of the endogenous peptides have been demonstrated to be released in increased amounts from nerve ending preparations subsequent to electrical stimulation or K^+ depolarization [8], we considered it relevant to determine whether the radio-labeled peptides which were available to us, ^3H-MIF-I, ^3H-α-MSH, and ^{14}C-AVP, are concentrated in nerve ending preparations by high affinity, Na^+ dependent, uptake mechanisms as has been demonstrated for neurotransmitter amines and amino acids [33,39].

In this report we also present data relative to: (a) the penetrability through the blood-brain barrier of the radio-labeled peptides; (b) the distribution of the radio-label in the major brain regions after penetration; and (c) the rate of disappearance of the radio-label from the major brain regions.

METHOD

Ninety male Holtzman rats (250–350 g) were used in the experiments. The radioisotopes used in the experiments were: [N-methyl-^{14}C] antipyrine (sp. act. 57 mCi/mM,

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Amersham/Searle Corp, Arlington Heights, Ill.); ^3H_2O (68 μCi/μl); [1-proline-2,3-^{3}H]MIF-I (sp. act. 4.9 Ci/mM) was used in early experiments and [1-proline-3,4-^{3}H]MIF-I (sp. act. 55.4 Ci/mM, RO-11-2028/7027) (^{3}H-MIF) in later experiments; [8-arginine, 9-glycinamide-1-^{14}C]vasopressin (sp. act. 30 mCi/mM) (^{14}C-AVP); and [2-tyrosine-3, 5-^{3}H] α-MSH (sp. act. 12 Ci/mM) (^{3}H-α-MSH).

The ^{3}H-α-MSH was prepared by catalytic tritiation of the 3,5-dibromotyrosine derivative of α-MSH as described by Kastin *et al.* [17] and the ^{14}C-AVP was from the same batch prepared by Walter and Havran [35]. The ^{3}H-MIF of the lower specific activity was prepared by using Boc-proline-2,3-^{3}H to complete the tripeptide by solid phase methodology [4]; the ^{3}H-MIF of higher specific activity was prepared by PdO-catalyzed tritiation of synthetic [3,4-dehydroproline^1]MIF-I in methanol by a modification of a previously published method (A.M. Felix, C. T. Wang, A. Liebman, C. M. Delaney and J. Meienhofer, manuscript in preparation) and was kindly supplied by Drs. J. Meienhofer and A. M. Felix of Hoffmann-LaRoche Inc., Nutley, N.J.

Intracarotid Artery Injection Experiments

A bolus of radio-labeled peptide was rapidly injected into the left common carotid artery of the rats in combination with either ^3H_2O or ^{14}C-antipyrine in order to determine the brain uptake index (BUI) of the whole brain as defined by Oldendorf [25,26] or of the major brain regions. The rats were anesthetized with ketamine hydrochloride (intramuscular injections of 66 mg/kg followed by 33 mg/kg 2-3 min later). After exposure of the left common carotid artery, a 0.2 ml bolus of the injectant consisting of one of the radio-labeled peptides and either ^3H_2O or ^{14}C-antipyrine as a counter-label in Kreb's-Ringer solution (composition in mEq/liter: Na^+, 147; K^+, 4; Ca^{++}, 2; Cl, 155) buffered at pH 7.56 with 4 mM N-2-hydroxy-ethylpiperazine N'-2-ethanesulfonic acid (HEPES, Calbiochem, La Jolla, Calif.) was administered with a 1 ml disposable syringe through a 27 gauge needle. The injection of the bolus was performed within 1 sec, care was taken not to impede the blood flow in the artery. The rats were decapitated with a guillotine either 15 sec or 10 min after the injection of the radio-labeled compounds. The brains were rapidly removed, blotted dry and weighed. For the BUI of whole brain, the entire brain was masserated by passing it twice through an 18 gauge needle attached to a 5 ml disposable syringe and 50-100 mg of tissue was weighed directly into scintillation vials. For the BUI of various brain regions, the brain was moistened with chilled saline (0.9% NaCl) and dissected over dry ice according to the method of Glowinski and Iverson [9]. The caudate nucleus and the hypothalamus were taken whole while the larger brain regions such as the cerebral cortex, the brainstem, and the cerebellum were masserated by passage through an 18 gauge needle attached to a 2 ml disposable syringe. Fifty to one-hundred mg of tissue from the various region were weighed and placed into scintillation vials. Two to four pineal or pituitary glands from each group of experiments were pooled and placed into scintillation vials. Fifty to one-hundred mg of brain tissue from uninjected rats were used for blank determination and for determining the exact amounts of radioactivity in the injectant solutions. In order to digest the brain tissue, 1 ml of NCS tissue solubilizer (Amersham/Searle Corp., Arlington Heights, Ill.) was added to each vial and left overnight at room temperature. The next morning the vials were incubated at 37°C for 3-4 hr, cooled to room temperature, and 12 ml of scintillation fluid added (5 g PPO, 0.05 g, POPOP in 1 liter toluene). In order to stabilize the scintillation system, the vials were kept in the dark and under refrigeration overnight. The vials were counted for 10 min in a Packard Model 3375 liquid scintillation spectrometer with windows set for ^{14}C/^{3}H dual counting system.

The dual isotopes injected in 0.2 ml buffer were as follows: (a) 4.5 μC ^{14}C-antipyrine/70-140 μC ^3H_2O; (b) 0.2-0.5 μC ^{14}C-AVP/70-140 μC ^3H_2O; (c) 8-14 μC ^{3}H-MIF-I/4.5 μC ^{14}C-antipyrine; and (d) 7 μC ^{3}H-α-MSH-I/4.5 μC ^{14}C-antipyrine.

Calculations

All data are expressed as the mean ± SEM and (n) represents the number of animals utilized to obtain the mean. ^3H_2O and ^{14}C-antipyrine were used to define the fraction of the injected bolus that entered the brain after intra-carotid injection. For ^{14}C-antipyrine and ^{14}C-AVP the brain uptake index relative to ^{3}H-water BUI_w were calculated according to Oldendorf [25]:

$$\frac{^{14}\text{C-tissue}/^{3}\text{H-tissue}}{^{14}\text{C-injectant}/^{3}\text{H-injectant}} \times 100 = BUI_w$$

For ^{3}H-MIF and ^{3}H-MSH the BUI_w relative to water was obtained by normalizing the experimentally obtained BUI_a relative to ^{14}C-antipyrine:

$$\frac{\text{Tissue } ^{3}\text{H-MIF}/^{14}\text{C-Antipyrine}}{\text{Injectant } ^{3}\text{H-MIF}/^{14}\text{C-Antipyrine}} \times 100 = BUI_a^{MIF}$$

$$[BUI_a^{MIF} \times (BUI_w^a \text{ of } ^{14}\text{C-Antipyrine}/^3H_2O)] \div 100 = BUI_w^{MIF}$$

The BUI_w^a of ^{14}C-antipyrine relative to water was 47.3 ± 2.4.

In Vitro Synaptosome Uptake Studies

Synaptosomes (S_1 fraction) from rat whole brain or from large brain regions were isolated using the technique described by Whittaker [38]. For the experiments in 143 mM Na^+, 0.2 ml of the S_1 fraction was incubated for 4 min at either 37°C or 4°C with varying concentrations of ^{3}H-MIF-I (4.7×10^{-10} - 1.0×10^{-5}M), ^{3}H-α-MSH (8.3×10^{-10} - 2.5×10^{-5}M) or ^{14}C-AVP (1.8×10^{-9} - 2.2×10^{-6}M) in 1.8 ml Kreb's-Ringer bicarbonate (pH 7.4) containing 1.1 mM ascorbic acid, 0.16 mM disodium-ethylenediamine-tetraacetic acid (EDTA, Fisher, Fair Lawn, N.J.) and 11.1 mM D-glucose as described by Kuhar, Roth and Aghajanian [19]. For the experiments with OmM Na^+ at 37°C the following changes were made: (a) tris (hydroxymethyl) aminomethane (Sigma, St. Louis, Mo.) was substituted for the bicarbonate buffer; (b) 0.32 M sucrose replaced the 0.9% saline in order to maintain the isotonicity of the incubation solution; (c) 0.16 mM ethylenebis (oxyethyleneitrilo)tetracetic acid (EGTA, Eastman, Rochester, N.Y.) which has K^+ as the cation replaced EDTA. After incubation with the respective peptide, the synaptosomes were collected and washed (using 10 ml of 0.9%

saline) on membrane filters with 0.45 μ pores (Millipore Corp., Bedford, Mass.) followed by H_2O vacuum filtration as described by Horn *et al.* [11]. The peptide-containing synaptosomes were eluted from the membrane filters by 12 ml of a scintillation cocktail containing Triton X-100 (5 g PPO, 0.9 g POPOP, 250 ml Triton X-100, and 750 ml toluene). Radioactivity in the samples were measured with a Packard model 3380 liquid scintillation spectrometer with an absolute activity analyzer.

The synaptosomal aliquots of the S_1 fraction contained 0.74–1.8 mg protein per incubation. The protein determinations were done according to the method of Lowry *et al.* [21].

RESULTS

Perfusion Experiments

The BUI values of ^{14}C-AVP and ^{14}C-antipyrine in comparison with ^3H_2O; and of ^{3}H-MIF and ^{3}H-α-MSH in comparison with ^{14}C-antipyrine were determined according to the method of Oldendorf [25,26]. For ease in presentation the data obtained with the ^{3}H-labeled peptides have been normalized in terms of peptide/H_2O as explained in the methods section. The normalized BUI values for the whole brain at 15 sec for the three peptides were similar: MIF-I, 13.7; α-MSH, 9.6; AVP, 13.0 (Table 1). The normalized BUI values for the major brain regions (Table 2) were similar to the whole brain BUI for the three peptides. The BUI values for the pooled pineal and pituitary glands (Table 2) were several fold larger with the MIF and MSH peptides while the BUI of AVP approached 100 in both glands. The normalized pineal gland BUI values were MIF-I, 23.2; α-MSH, 23.5; AVP, 93.5. The normalized pituitary gland BUI values were: MIF-I, 40.9; α-MSH, 39.4; AVP, 91.4. The % label of the originally injected peptides recovered/g whole brain was 0.5, 0.6 and 0.5 for the ^{3}H-MIF-I, ^{3}H-α-MSH and ^{14}C-AVP, respectively, as recorded in Table 1. The % recovery per g tissue of the radio-labeled peptides in the pooled pineal and pituitary glands were substantially increased corresponding to the increased BUI values.

In other experiments the BUI values and the % of the originally injected dose of two of the radio-labeled peptides (MIF-I, AVP) (we did not have a sufficient amount of ^{3}H-α-MSH to do additional experiments) were determined at 10 minues subsequent to the intra-carotid injection of labeled material. The normalized BUI values obtained for the whole brain (Table 1) and the major brain regions (Table 2) at 10 min were little changed from the values obtained at 15 sec.

In contrast, the normalized BUI values obtained for the pooled pineal and the pooled pituitary glands were significantly increased 10 min after injection (Table 2). Furthermore, the % of the originally injected dose per gram of glandular tissue was further increased corresponding with the increased BUI values; this is consistent with sequestration of peptides by these glands.

The % label of the originally injected dose of the radio-labeled peptides recovered per g tissue in the whole brain (at 10 min after injection) is recorded in Table 1: ^{3}H-MIF-I, 0.2%; ^{14}C-AVP, 0.1%. The % remaining of the radio-labeled peptides and either the ^3H_2O or ^{14}C-AVP was reduced proportionally since the BUI values at 15 sec and 10 min were similar. There was little evidence of sequestration by the whole brain or the major brain regions of ^{3}H-MIF-I or ^{14}C-AVP as compared with ^{14}C-antipyrine and ^3H_2O, respectively in contrast to the 10-20x increased sequestration of both peptides by the pineal and pituitary glands (Table 2).

Uptake of Peptides by Synaptosomes

The uptake of ^{3}H-MIF-I, ^{3}H-α-MSH and ^{14}C-AVP in pm × 10^4/mg protein/4 min by synaptosomes prepared from rat brain and incubated in vitro at 37° C and 4° C in 143 mM and 0 mM Na^+ is presented in Fig. 1. The uptake of each of the peptides is unchanged at 37° and at 4°, in the presence or absence of 143 mM Na^+, compatible with uptake by passive diffusion. However, there is a 50–100x increased uptake of ^{14}C-AVP and a several fold increased uptake of ^{3}H-α-MSH as compared with ^{3}H-MIF-I.

The uptake of ^{3}H-MIF-I and ^{14}C-AVP at 37° C and 4° C in 143 mM Na^+ and 0 mM Na^+ by synaptosomes prepared from the cerebral cortex, hypothalamus and forebrain is also consistent with passive uptake (Fig. 2). The values for a given peptide were similar for the major brain regions; there was no clear indication of active uptake or preferential uptake by the synaptomsomes of any of the brain regions. However, as with the whole brain synaptosomes there is a 50–100 x increased uptake of ^{14}C-AVP on a molar basis as compared with ^{3}H-MIF-I.

In two additional experiments we determined the ^{14}C-AVP uptake by a modified procedure. We incubated 3–5 additional aliquots of whole brain synaptosomes at two representative concentrations of ^{14}C-AVP (4×10^{-8}, 4

TABLE 1

BRAIN UPTAKE INDEX OF LABELED PEPTIDES AT 15 SEC AND 10 MIN AFTER INTRA-CAROTID INJECTION

Peptide	BUI		% Injected Label/g Tissue	
	15 sec	10 min	15 sec	10 min
^{14}C-Antipyrine	47.3 ± 2.4 (5)	57.0 ± 6.5 (5)	4.5 ± 0.4 (10)	0.4 + 0.1 (5)
^{3}H-MIF-I	13.7 ± 1.6 (15)	11.3 ± 2.7 (5)	0.5 ± 0.1 (15)	0.2 ± 0.1 (8)
^{3}H-α-MSH	9.6 ± 2.3 (5)	—	0.6 ± 0.1 (4)	—
^{14}C-AVP	13.0 ± 2.8 (8)	24.7 ± 9.8 (5)	0.5 ± 0.1 (8)	0.1 ± 0.1 (5)

BUI for ^{3}H-MIF-I and ^{3}H-α-MSH were found experimentally in relation to ^{14}C-Antipyrine and converted to a peptide/water normalized value using the BUI of ^{14}C-Antipyrine/^3H_2O as described in the methods section. The amount of the injected labels were as follows: ^3H_2O = 70-140 μC, ^{14}C-Antipyrine = 4.5 μC, ^{3}H-MIF-I = 0.14-2.86 × 10^{-3} μmoles, ^{3}H-α-MSH = 5.83 × 10^{-3} μmoles and ^{14}C-AVP = 6.7-16.7 × 10^{-3} μmoles. The numbers in brackets represent the number of animals used. The data are expressed as the x ± SEM.

TABLE 2

UPTAKE OF LABELED PEPTIDES INTO LARGE BRAIN REGIONS

Peptide	Region	Uptake 15 sec	Uptake 10 min	% Injected Label/g Tissue 15 sec	% Injected Label/g Tissue 10 min
^{3}H-MIF-I	C. Cortex	10.1 ± 2.3 (5)	15.1 ± 3.7 (3)	0.41 ± 0.14 (5)	0.10 ± 0.03 (3)
	Hypothalamus	10.1 ± 2.3 (5)	12.5 ± 1.9 (3)	0.29 ± 0.07 (5)	0.09 ± 0.02 (3)
	Caudate	8.8 ± 1.4 (5)	13.7 ± 3.2 (3)	0.28 ± 0.07 (5)	0.09 ± 0.03 (3)
	B. Stem	11.5 ± 3.5 (5)	14.3 ± 0.6 (3)	0.10 ± 0.02 (5)	0.09 ± 0.01 (3)
	Cerebellum	13.6 ± 4.2 (5)	16.0 ± 2.1 (3)	0.29 ± 0.10 (5)	0.10 ± 0.03 (3)
	Pineal	23.2 ± 4.5 (2)	198 (1)	3.65 ± 1.25 (3)	2.00 (1)
	Pituitary	40.9 ± 2.8 (2)	52 (1)	8.30 ± 1.95 (3)	1.10 (1)
^{3}H-α-MSH	C. Cortex	10.3 ± 3.4 (3)		0.49 ± 0.09 (3)	
	Hypothalamus	12.5 ± 5.8 (3)		0.58 ± 0.28 (3)	
	Caudate	11.0 ± 3.8 (3)		0.61 ± 0.23 (3)	
	B. Stem	14.7 ± 2.0 (3)		0.17 ± 0.03 (3)	
	Cerebellum	13.4 ± 3.0 (3)		0.22 ± 0.03 (3)	
	Pineal	23.5 (1)		0.75 (1)	
	Pituitary	39.4 (1)		8.35 (1)	
^{14}C-AVP	C. Cortex	14.6 ± 4.0 (5)	24.7 ± 9.8 (5)	0.49 ± 0.15 (6)	0.13 ± 0.05 (5)
	Hypothalamus	14.7 ± 3.7 (6)	14.9 ± 4.8 (5)	0.63 ± 0.18 (6)	0.08 ± 0.03 (5)
	Caudate	12.2 ± 3.8 (6)	17.1 ± 5.4 (5)	0.64 ± 0.13 (6)	0.09 ± 0.04 (5)
	B. Stem	18.0 ± 3.6 (6)	20.6 ± 5.6 (5)	0.39 ± 0.08 (6)	0.09 ± 0.03 (5)
	Cerebellum	16.0 ± 3.7 (6)	19.4 ± 7.3 (5)	0.38 ± 0.11 (6)	0.11 ± 0.04 (5)
	Pineal	93.5 ± 14.6 (2)	160 (1)	3.80 ± 0.1 (2)	0.70 (1)
	Pituitary	91.4 (1)	117 (1)	7.80 (1)	1.40 (1)

The animals were sacrificed at either 15 sec or 10 min following the intracarotid injection of the labeled peptides. Uptake of ^{3}H-MIF-I and ^{3}H-α-MSH were found experimentally in relation to ^{14}C-Antipyrine and converted to a peptide/water normalized value using the BUI of ^{14}C-Antipyrine/$^{3}H_2O$ as described in the methods section. See Table 1 for μcuries injected. The numbers in brackets represent the number of animals used, except for values for the pineal and pituitary glands for which 2-4 glands were pooled per determination. In the latter case the number in brackets represents the number of determinations with pooled glands. The data are expressed as the x ± SEM.

$\times 10^{-9}$ M) at 37° and 4° C and harvested the synaptosomes by low speed centrifugation rather than by the usual millipore filter procedure. The control pellets were compared with the experimental pellets which were lysed in 3 ml of 4° C water with mixing and recentrifuged to yield a supernatant and membrane fraction. All of the ^{14}C-AVP label was recovered in the membrane fraction in comparison with the control synaptosomes. Almost no label was recovered in the supernatant.

DISCUSSION

We have demonstrated by means of the Oldendorf procedure [25,26] that there is an uptake of ^{3}H-MIF-I, ^{3}H-α-MSH and ^{14}C-AVP in brain sufficient to give a BUI between 9.6 and 13. A BUI of 1.4–2.3 is interpreted as the background level compatible with the amount present in the brain circulation and in the endothelium. Since the BUI for these three peptides is more than 3x above background, we interpret this to mean that substantial amounts of the peptides have passed the blood-brain barrier and entered the brain tissue.

Similarly, since the % recovery of the injected label (in the brain) at 15 sec is about 3 x greater than the % recovery at 10 min we interpret this to mean that most of the peptide present at 15 sec has been cleared from the brain at 10 min.

Previously DuPont *et al.* [6,7] demonstrated high levels of radioactivity in the pineal and in the anterior and posterior pituitary regions of rats and mice by whole body radioautography and by direct measurement of gland homogenates at 2 min and 10 min after the IP injection of radio-labeled MIF-I and α-MSH while at the same time there was a zero or negligible level of ratio-activity in the radioautograph of the rest of the brain (however, they recovered appreciable amounts of radioactivity in the homogenates of the same brain regions).

In other experiments, Pelletier *et al.* [29,30] observed a low uptake of radioactivity in tissue sections of rat brain at 5 min after the intracarotid injection of larger doses of MIF-I and α-MSH. The uptake was highest in the areas surrounding the blood vessels and in the choroid plexus and ependymal cells; it was concluded that the label penetrated the blood-brain barrier and perhaps the choroid-CSF barrier prior to its localization in several specific brain areas.

Based on our BUI data the low levels of radioactivity observed by DuPont *et al.* [6,7] and Pelletier *et al.* [29,30] were a consequence of the rapid rate of egress of the ^{3}MIF-I and ^{3}H-α-MSH label at 10 min and at 5 min after injection.

In correspondence with the data of DuPont *et al.* [6,7], we obtained high BUI values for ^{3}H-MIF-I and ^{3}H-α-MSH in the pineal at 15 sec and even higher BUI values for ^{3}H-MIF-I at 10 min. The % of the injected ^{3}H-MIF-I/g of tissue was increased roughly 10–20x in the pineal and pituitary tissues at 15 sec and at 10 min in comparison with

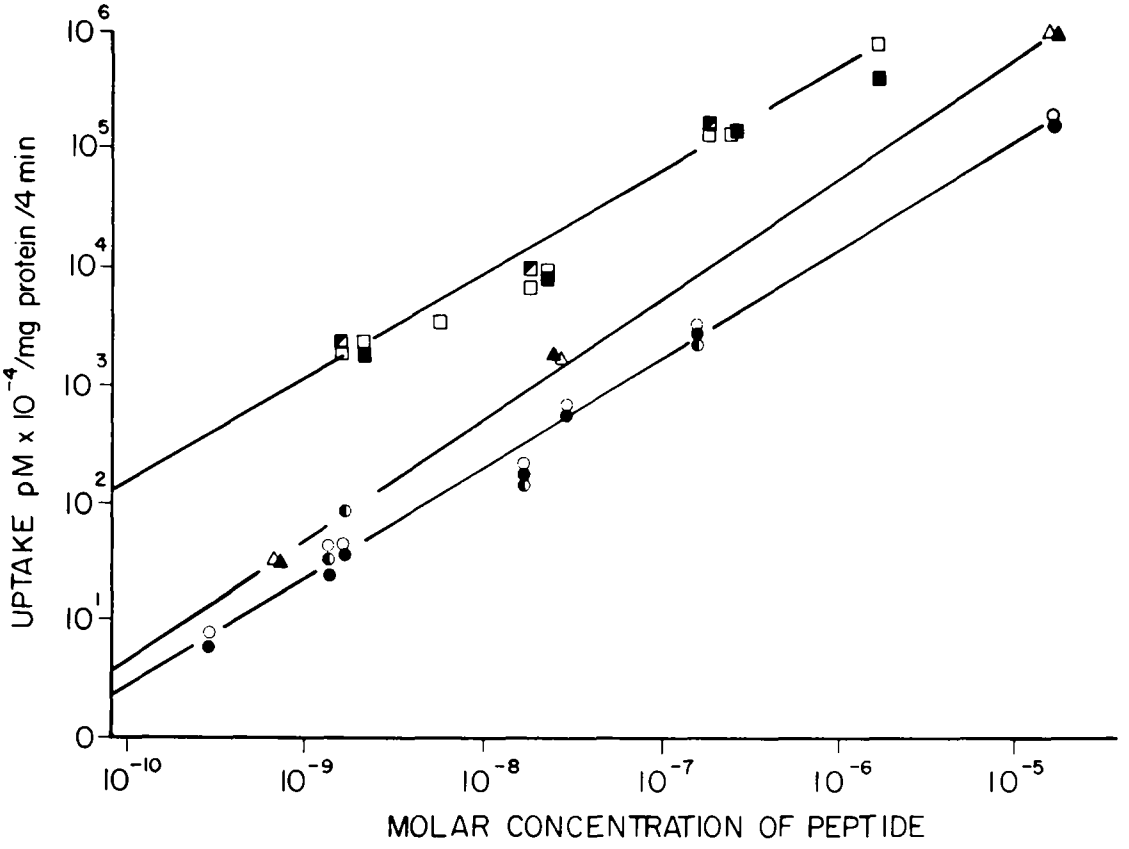

FIG. 1. The in vitro uptake of ^{3}H-MIF-I, ^{3}H-α-MSH, and ^{14}C-AVP by synaptosomes (S_1 fraction) prepared from rat brain. The synaptosomes were incubated at 37°C and at 4°C in Kreb's-Ringer bicarbonate (pH 7.4) containing 143 mM Na^+ or Kreb's-Ringer containing 0.018 M Tris buffer (pH 7.4 at 37°C) for the experiments with 0 mM Na^+. The interpretation of the symbols is as follows: ○ = ^{3}H-MIF-I, 143 mM Na^+, 37°C; ● = ^{3}H-MIF-I, 143 mM Na^+, 4°C; ◐ = ^{3}H-MIF-I, 0 mM Na^+, 37°C; △ = ^{3}H-α-MSH, 143 mM Na^+, 37°C; ▲ = ^{3}H-α-MSH, 143 mM Na^+, 4°C; □ = ^{14}C-AVP, 143 mM Na^+, 37°C; ■ = ^{14}C-AVP, 143 mM Na^+, 4°C; ◩ = ^{14}C-AVP, 0 mM Na^+, 37°C. Each point represents the mean of 4–14 determinations. The statistical variations were determined but not illustrated. The SEM values were less than ± 5%. The differences at 37°C versus 4°C and 143 mM Na^+ versus 0 mM Na^+ were not significant.

the major brain regions. Presumably, there are more binding sites for peptides in general since the BUI values and the % recovery values were similarly increased for ^{14}C-AVP. Alternatively, the increased pineal and pituitary uptake values are a consequence of their being outside the blood-brain barrier [32, 37, 42], a barrier which diminishes the penetrability of the peptides to give a BUI of about 10 in comparison with ^3H_2O taken as 100.

The uptake by the whole brain and by the major brain regions of about 0.5% of the injected label at 15 sec and 0.1–0.2%/g of the injected label at 10 min after injection is coincident with a relatively unchanged BUI at 15 sec and 10 min. It would seem, therefore, the peptides are able to enter and leave at the same rate as the ^3H_2O and ^{14}C-antipyrine counterlabels. Presumably the peptide label is distributed uniformly in the brain regions except the lower brain stem and the cerebellum which receive a portion of their arterial blood via the vertebral artery [23] and therefore have BUI values that are slightly decreased. Kastin *et al.* [17] observed more ^{3}H-α-MSH in occipital cortex, cerebellum and pons-medulla than in other major brain regions particularly after 15 sec although the Oldendorf procedure was not used.

The half-time of disappearance of the peptides from rat plasma after IV injection is short. The half-time disappearance of radioactivity from the plasma of rats of synethetic MIF-I, α-MSH and AVP are of the order of 9 min [31], 2.5 min [17], and 1.5 min [5] respectively, and presumably some of the unchanged peptides would be re-perfused in sufficient amounts to maintain about 0.1–0.2%/g of brain of the injected label in a brain which comprises about 1% of the body weight.

If the endogenous concentrations of these peptides is unequal in the major brain regions, it would not appear to be a function of the arterial perfusion; but rather it would be related to variations in metabolism or uptake or, perhaps local biosynthesis of the respective peptides in specific brain regions.

We did not determine whether the peptides were intact in the brain homogenates at 15 sec and 10 min. Almost certainly, the peptides were not degraded in the carotid arterial blood and were intact when they penetrated the blood-brain barrier and at 15 sec after the injection. Probably, they were in part degraded by 10 min after the injection. Redding *et al.* [31] recovered 9–26% of the radioactivity of ^{14}C-leucine-labeled MIF-I as unchanged peptide in rat pineal and pituitary extracts at 1 hour after in vivo injection. Similarly, Kastin, *et al.* [17] recovered a

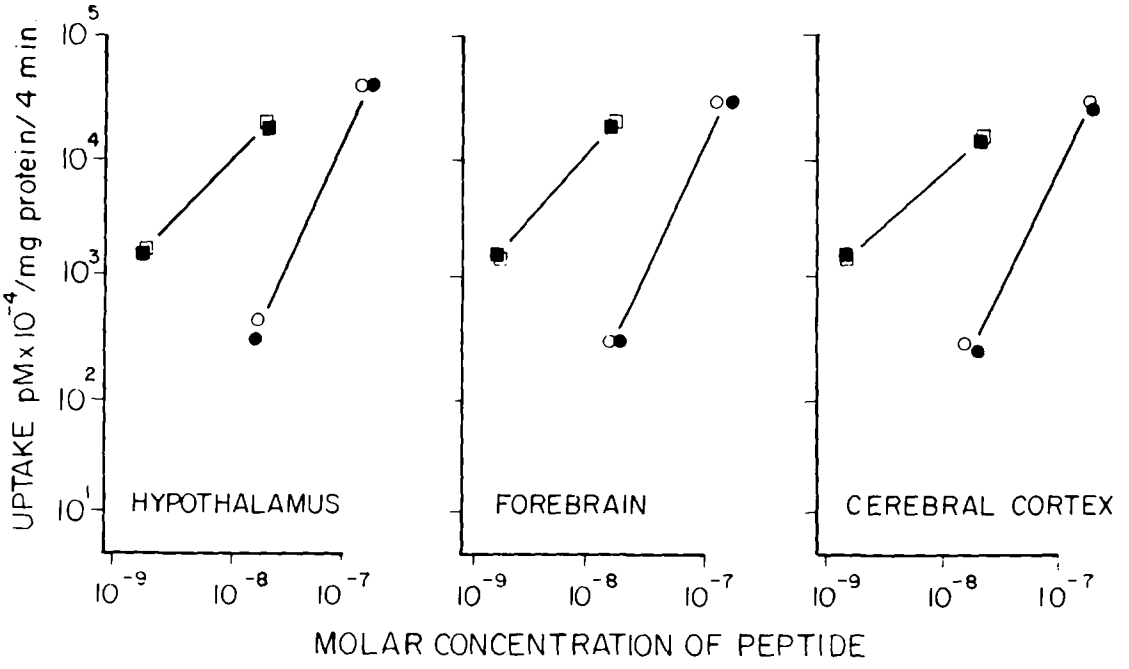

FIG. 2. The in vitro uptake of ^{3}H-MIF and ^{14}C-AVP by synaptosomes (S_1 fraction) prepared from various brain regions of rat brain. The synaptosomes were incubated at 37°C and 4°C in Kreb's-Ringer bicarbonate (pH 7.4) containing 143 mM Na^+. The interpretation of the symbols is given in Fig. 1. The statistical variations were determined, but not illustrated. The SEM values were less than ± 5%. The differences at 37°C versus 4°C were not significant.

part of the radioactivity of ^{3}H-α-MSH as unchanged peptide in the rat brain as late as 30 min after in vivo injection.

Based on the rapid rate of egress of the ^{3}H-MIF-I, it is probably not degraded to the constituent ^{3,4}ditritio proline which would exit slowly in correspondence with a BUI of 3.5 as reported by Oldendorf [26]. The ^{3}H label of MSH was on the tyrosine and the ^{14}C label of AVP was on the glycine; if either of these amino acids were produced in the brain as enzymatic degradation products, then the tyrosine would readily depart the brain and the glycine would not, in correspondence with the BUI values of tyrosine and glycine at 50 and 2.5, respectively, as reported by Oldendorf [26].

The in vitro uptake of the three peptides by the synaptosomes (S_1 fraction) prepared from the whole brain (Fig. 1) and from the major brain regions (Fig. 2) is consistent with passive uptake: there was no metabolic requirement; there was no Na^+ requirement; there was no preferential uptake by the synaptosomes of any one of the large brain regions. However, there was a 50–100 x increased uptake of ^{14}C-AVP and a several fold increased uptake of ^{3}H-α-MSH as compared with ^{3}H-MIF-I on a molar basis.

Presumably the great majority of the uptake of the three peptides is based on nonspecific binding which greatly exceeds any receptor binding which exists. The concentrations of MIF-I and α-MSH per g of synaptosome, based on the protein content as 13% of wet weight, was not increased above the medium concentration; however, there was 3–10 x increased concentration of AVP in the synaptosomes in comparison with the incubate and almost all of the AVP label was associated with the membranes subsequent to lysis of the synaptosomes in cold water.

We do not offer an explanation for the marked differences in binding. The temperature dependence of the binding at 37°C and 4°C was smaller than the experimental error. This permits us to set the upper limit of the binding energy as 2 Kcal/mol which is of the order of magnitude of Van der Waals' interactions.

The pharmacologic or extra-endocrine effects of MIF, MSH and AVP upon the brain and behavior have been reviewed recently [14, 16, 40]; clearly all three of the peptides produce CNS behavioral effects over a period of several hours after parenteral administration. The iontophoretic application of AVP to selected neurons of the mollusc produces neurotransmitter-like effects (at high concentrations) and neuro-modulating effects (at lower concentrations) lasting during several hours of washing in peptide-free medium [2]. Long lasting effects have been observed with the administration of substance P on cuneate neurons in mammals [18]; and MIF and MSH may also have neuromodulatory effects. Based on our in vitro synaptosome uptake studies it is unlikely that the action of AVP (or MIF and MSH) is terminated by re-uptake into synaptosomes; rather, based on our carotid artery perfusion studies, wherein only a minor fraction of the radio-label is recoverable at 10 min, it would appear that the remaining minor fraction (almost all of which is associated with the membrane) is in equilibrium with extracellular fluids or is slowly metabolized. Although the radio-labeled peptides were distributed uniformly in the major brain regions after the intracarotid injections, it is likely that minute amounts of the peptides were taken up selectively by nerve cells as has been shown for MSH and MIF [29,30] or by nerve endings as has been shown for several other peptides [27,28].

An interaction between melatonin, MIF, and MSH wherein each component of the pineal, hypothalamic,

pituitary axis affects every other has been proposed [15]. It is possible that AVP (this paper), and perhaps many other peptides, might be part of the interaction. Recently, we have observed a similar increased concentration of phenylethylamine in the pineal and pituitary glands after intracarotid injection [10], and thus circulating amines might also be part of the interaction.

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