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A Brain-to-Blood Carrier-Mediated Transport System for Small, N-Tyrosinated Peptides

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BANKS, W. A. AND A. J. KASTIN. *A brain-to-blood carrier-mediated transport system for small, N-tyrosinated peptides*. PHARMACOL BIOCHEM BEHAV **21**(6) 943–946, 1984.—Recently, we found that lipophilicity is a good predictor of the degree to which most peptides cross the blood-brain-barrier. Small (MW<1000) peptides with an N-terminal tyrosine, however, penetrated to a much smaller degree than was predicted by their measurements of lipophilicity. We show here that two such peptides, N-Tyr-MIF-1 and Met-enkephalin, can significantly inhibit transport of ^{125}I-N-Tyr-MIF-1 out of the rat brain *in vivo* in a saturable, dose-dependent way. The half-time disappearance of injected ^{125}I-N-Tyr-MIF-1 from the rat brain was 12.4 min but when injected with 200 nmol/animal of unlabeled N-Tyr-MIF-1 was 23.6 min ($p<0.01$). The Km was calculated to be 0.123 nmol. At higher doses, leucine, but not tyrosine, alanine, glutamine, MIF-1, or the dipeptide Gly-Gly, also significantly inhibited transport out of the brain.

Blood-brain-barrier Peptide(s) Brain Tyr-MIF-1 Cerebrospinal fluid Carrier-mediated transport Enkephalin(s)

IT is well established that peripheral administration of neuropeptides causes behavioral and central nervous system (CNS) effects [12]. There are probably several mechanisms by which peptides exert these effects, including interaction with receptors at the circumventricular organs [4], mediation via vagal afferent fibers [15,21], modulation of blood-brain barrier (BBB) permeability to non-peptide substances [19, 20, 22], and direct penetration of peptide substances across the brain.

Several studies have shown that peptide substances can cross the BBB in intact form [2, 3, 6, 7, 13, 16]. The degree of penetrance varies with different peptides and their analogs [2, 10, 18]. In general, transport seems to be by a non-saturable mechanism [1]. Recent studies have shown that the degree to which most peptides cross the BBB can be predicted from their physicochemical properties, especially their lipophilicity (Banks and Kastin, submitted). Some peptides, however, appeared to cross to a much smaller degree than would be predicted by their lipophilicity. In general, these compounds are characterized by low molecular weight (less than 1000), high lipophilicity, slight molecular charge, and an N-terminal tyrosine. This group includes N-Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2), Met-enkephalin (Tyr-Gly-Gly-Phe-Met), and Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu). The occurrence of such a group of compounds raises the question of a brain-to-blood carrier-mediated transport system for these peptides. Such a system could lead to the rapid egress of these peptides from the brain, thus resulting in the false appearance of decreased entry into the CNS.

METHOD

Peptides were synthesized by solid phase methods. N-Tyr-MIF-1 was iodinated (^{125}I) with the use of chloramine-T and subsequent purification on a column of Sephadex G-15. 250,000 CPM of iodinated N-Tyr-MIF-1 with or without 200 nanomoles (nmol) of unlabeled N-Tyr-MIF-1 was injected in a volume of 10^{-2} ml 0.9% NaCl into the right lateral ventricle of 200 g rats anesthetized with sodium pentobarbital (65 mg/kg, IP) using the technique described by Noble [17]. The rats were decapitated at 1 min, 5 min, 10 min, 20 min, and 30 min after injection and the whole brain counted in a gamma counter for 3 min.

The effect of various concentrations of unlabeled N-Tyr-MIF-1 on transport of labeled N-Tyr-MIF-1 out of the brain was also studied and expressed as percent inhibition. The percent inhibition (%I) was calculated for animals decapitated 10 min after injection by the equation:

$$\%I = (\dot{P} - C)/(10^5 - C)$$

where C represents the mean counts per whole brain in animals injected with iodinated N-Tyr-MIF-1 only and P the counts per whole brain in individual animals injected with both iodinated N-Tyr-MIF-1 and unlabeled competitor. The value of 10^5 was used in the denominator since this was the number obtained by extrapolation to time zero of the data describing the transport of iodinated N-Tyr-MIF-1 out of the brain.

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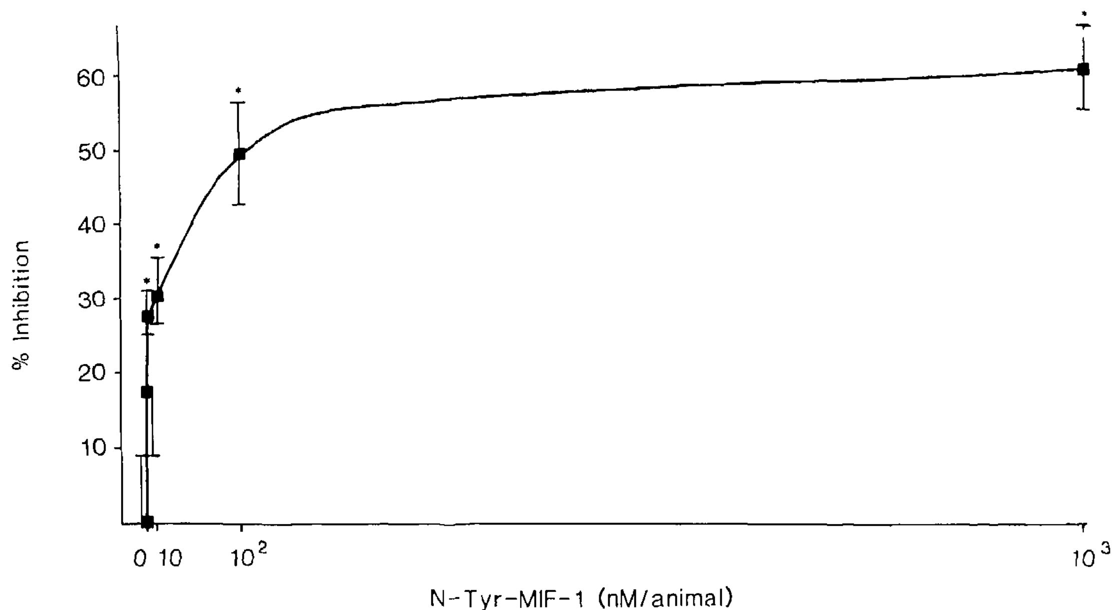

FIG. 1. Inhibition of exit of ^{125}I-N-Tyr-MIF-1 from the brain by increasing amounts of N-Tyr-MIF-1 (nmol/animal).

TABLE 1

DISAPPEARANCE OF IODINATED N-TYR-MIF-1 FROM THE BRAIN OVER TIME AND ITS INHIBITION BY 200 NMOL/ANIMAL OF UNLABELED N-TYR-MIF-1

Time (min)	^{125}I-N-Tyr-MIF-1 (CPM/Brain)	N-Tyr-MIF-1 + ^{125}I-N-Tyr-MIF-1 (CPM/Brain)
1	123,429 ± 13,764	116,094 ± 2,697
5	70,195 ± 1,901	102,645† ± 8,709
10	52,615 ± 3,771	78,854* ± 9,418
20	42,520 ± 5,992	68,443* ± 8,782
30	20,757 ± 4,313	47,435* ± 5,024

*$p<0.05$ or †$p<0.01$ compared with value at same time point (without unlabeled peptide).

Other substances were tested at varying concentrations for competition. Methionine enkephalin (Met-Enk), MIF-1 (Pro-Leu-Gly-NH_2), tyrosine, leucine, and alanine were tested at the dose of 1000 nmol/rat and 100 nmol/rat, while the dipeptide Gly-Gly and glutamic acid were tested at the dose of 1000 nmol/rat only. Results were expressed as %I.

Duncan's multiple range test preceeded by an ANOVA was performed when comparisons were made among populations. Regression coefficients, slopes, and intercepts were determined by the least squares method.

RESULTS

The results (Table 1) show that N-Tyr-MIF-1 inhibited the disappearance of iodinated N-Tyr-MIF-1 from the brain, $F(4,26)=19.3$, $p<0.0005$. Significant differences occurred at 5 min ($p<0.01$), 10 min ($p<0.05$), 20 min ($p<0.05$), and 30 min ($p<0.05$). The half-time disappearance in rats injected with only iodinated peptide was 12.4 min as compared to 23.6 min for those whose injection also contained unlabeled material. With a one compartment model and zero order kinetics, the equation describing $\log_{10}$ CPM/whole brain (Y) vs. time (x) was $Y = -2.44(10^{-2})\ x + 5.00$ for animals given only iodinated peptide and $Y = -1.28(10^{-2})\ x + 5.07$ for animals that were also given non-iodinated material. The calculated concentration in the brain at time 0 was thus 100,000 CPM/whole brain for animals injected with iodinated peptide only. ANOVA of the regression coefficients showed that these equations were statistically different, $F(2,6)=13.3$, $p<0.01$.

As shown in Fig. 1, concentrations of 1, 10, 100, and 1000 nmol/animal, but not 0.1 nmol/animal, resulted in a significant inhibition of peptide transport. The equation describing the inverse plot of the reciprocals was: $1/Y = 3.12\ (10^{-3})\ 1/x + 0.0254$, giving a Km of 0.123 nmol.

Substances other than N-Tyr-MIF-1 were tested as inhibitors. Alanine. tyrosine, MIF-1, glutamine, and the dipeptide Gly-Gly did not cause significant inhibition at the concentrations tested. Only N-Tyr-MIF-1 and Met-enkephalin caused significant inhibition at the 100 nmol/animal concentration and N-Tyr-MIF-1, Met-enkephalin, and leucine at the 1000 nmol/animal concentration. The inhibition produced by Met-enkephalin was statistically significant when compared with tyrosine ($p<0.001$) or MIF-1 ($p<0.01$). Likewise, the inhibition produced by N-Tyr-MIF-1 was statistically significant when compared with tyrosine ($p<0.05$), and tended towards significance ($p=0.08$) when compared with MIF-1.

DISCUSSION

The results show that a competitive form of transport directed from the brain to the blood exists in which two biologically active peptides with tyrosine at the N-terminus (Met-enkephalin and N-Tyr-MIF-1) are active. Previous

TABLE 2

PERCENT INHIBITION BY VARIOUS SUBSTANCES ON THE TRANSPORT OF IODINATED N-TYR-MIF-1 OUT OF THE BRAIN

Dose (nmol/rat)	0.9% NaCl	Met-Enk	Tyr-MIF-1	MIF-1	Gly-Gly	Glu	Tyr	Leu	Ala
1000	0.19 ± 5.0	120* ± 24	81.7* ± 10	39.2 ± 9.4	0.39 ± 14	−7.2 ± 15	24.6† ± 11	91.4* ± 21	46.5 ± 22
100	0.06 ± 9.4	40.1* ± 11	53.3* ± 14	27.8 ± 20	NT	NT	28.5 ± 11	26.4 ± 12	−3.9 ± 5.7

*= $p<0.05$ compared with the control of 0.9% NaCl, †=injected as suspension, NT=not tested.

studies have shown that the octanol coefficient, a measure of lipophilicity [14], can be a good predictor of BBB penetrance for most iodinated peptides (Banks and Kastin, submitted). A subgroup of peptides, however, was found with penetrance values far below those predicted by their octanol coefficients. The most striking similarity among peptides comprising this subgroup was small size (MW<1000) in combination with an N-tyrosine. The existence of such a subgroup, that includes the enkephalins and N-Tyr-MIF-1, an endogenous tetrapeptide [11,23] with opiate antagonistic actions [9], raised the possibility that alternate means of penetrance might occur. A specific transport system out of the brain for these peptides was considered. The results of these studies provide strong evidence for such a system.

The half-time disappearance from the brain of ^{125}I-N-Tyr-MIF-1 was prolonged in the presence of unlabeled N-Tyr-MIF-1. Amounts of unlabeled peptide as small as 1 nmol/animal caused statistically significant inhibition of transport, and saturation appears to have been achieved between 100 and 1000 nmol/animal. The system exhibits specificity in that, of the compounds tested, only Met-enkephalin and N-Tyr-MIF-1 inhibited transport at both concentrations. Inhibition by Met-enkephalin was statistically significant when compared with either tyrosine ($p<0.001$) or MIF-1 ($p<0.01$). The effects of N-Tyr-MIF-1 were significant when compared with tyrosine ($p<0.05$), but missed significance when compared with MIF-1 ($p=0.08$). Inhibition by leucine was not competitive at the 100 nmol/animal concentration. This suggests a hierarchy in peptide specificity in which the presence of an N-tyrosine, although of great importance, may not be the only contributory factor.

Previous studies conducted *in vitro* have suggested that a saturable transport system exists in the choroid plexus for DAME, an analog of Met-Enk [5,8]. Because of the nature of these studies, it was not possible to determine if peptide was transported from the brain to the blood or from the blood to the brain. The system described here appears to be different from the one described *in vitro* in that tyrosine was not able to inhibit transport in our system, although it was able to do so in the *in vitro* DAME system. Our study also illustrates that transport from both directions must be examined before premature conclusions are drawn that peptides such as the enkephalins do not cross the BBB.

It is concluded, therefore, that some small, behaviorally active peptides can be transported out of the brain by a saturable, carrier-mediated process. For the particular process studied, an N-terminal tyrosine appears to have conferred specificity on the transport mechanism.

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