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DIFFERENTIAL METABOLISM OF Tyr-MIF-1 AND MIF-1 IN RAT AND HUMAN PLASMA

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Abstract—The metabolism of the endogenous brain peptides Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) and MIF-1 (Pro-Leu-Gly-NH_2) was determined by HPLC after incubation of the tritiated peptides in human and rat plasma. Degradation of Tyr-MIF-1 was rapid in the plasma from both species, in contrast to the slightly delayed degradation of MIF-1 in rat plasma and the extremely prolonged persistence of MIF-1 in human plasma. In rat plasma, more than half of the intact Tyr-MIF-1 and MIF-1 was degraded within 5 min, in contrast to the 5 days required for 50% degradation of MIF-1 in human plasma at 37°. To slow the rapid rate of metabolism, studies were then performed at 0°. Incubation of Tyr-MIF-1 in human plasma at 0° for 2 hr resulted in HPLC identification of more Tyr-Pro than Tyr at all times. At 0° in rat plasma, however, more Tyr than Tyr-Pro was formed after the first 5 min of incubation of the Tyr-MIF-1 that was labeled on the Tyr. This raised the possibility that the tetrapeptide Tyr-MIF-1 might be serving as a precursor of the tripeptide MIF-1. Incubation of Tyr-MIF-1 tritiated at the Pro under the same conditions with and without Tyr-MIF-1 tritiated at the Tyr showed that Tyr-Pro, not MIF-1, was the predominant degradation product of Tyr-MIF-1. In addition to the metabolism of Tyr-MIF-1 being slower at lower temperatures, it was also slowed by some enzyme inhibitors. After 10 min of incubation at 37°, EDTA appeared to be more effective than bestatin, *p*-chloromercuribenzoic acid (PCMB), pepstatin, or aprotinin, but after 30 min, bestatin was more effective. Intravenous injection of the tritiated peptides into rats showed short half-time disappearances; again, MIF-1 persisted in blood longer than Tyr-MIF-1. Thus, the results show the rapid metabolism of Tyr-MIF-1 in human and rat plasma, the slightly slower metabolism of MIF-1 in rat plasma, the predominant formation of Tyr-Pro rather than MIF-1 from Tyr-MIF-1, and the markedly delayed metabolism of MIF-1 in human plasma.

Key words: Tyr-MIF-1; MIF-1; metabolism; rat; human; HPLC

MIF-1† (Pro-Leu-Gly-NH_2) and Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) are endogenous [1–3] brain peptides that can exert biological actions including opiate modulation and antidepressant effects in rats [4–11] and humans [12–14]. Some of the differences between these compounds are that Tyr-MIF-1 but not MIF-1 (a) can bind in high concentrations to mu opiate receptors [15, 16], (b) can be transported from the central nervous system to blood by PTS-1 [17, 18], (c) can exert opiate as well as antiopiate effects in the guinea pig ileum assay [19], and (d) can decrease PKA activity in cultured cells [20].

Early studies of the metabolism by rats and humans of MIF-1 showed that there were major differences between the species. One hour after the rapid intravenous administration of labeled MIF-1 in the rat, no intact peptide could be found in the urine [21], whereas in humans more than 70% of the peptide recovered from urine was found as the intact molecule [22]. Similarly, large differences between the metabolism of MIF-1 in rat and human plasma have been found *in vitro* [23, 24].

In brain tissue from rats, it is controversial whether MIF-1 is a metabolic product of oxytocin [25, 26], but there is a report that this tripeptide can be formed from Tyr-MIF-1 [27]. The metabolism of Tyr-MIF-1 in human or rat plasma has not been reported, so it is not known whether the large difference in the metabolism of MIF-1 between rat and human plasma also applies to Tyr-MIF-1 or whether Tyr-MIF-1 is the precursor of MIF-1 in plasma.

MATERIALS AND METHODS

Tyr-MIF-1 was labeled with tritium by catalytic reduction of the precursor peptide iodinated with ^{127}I on the Tyr (Amersham, Arlington Heights, IL). MIF-1 was labeled by coupling tritiated BOC-Pro to Leu-Gly-NH_2 and then removing BOC (New England Nuclear, Boston, MA). The Tyr-MIF-1, labeled on the Tyr, was used for all experiments except where the use of Tyr-MIF-1 labeled on the Pro is indicated. The proline-labeled Tyr-MIF-1 was tritiated (Amersham) from the [dehydro Pro]^2 Tyr-MIF-1 synthesized in our laboratory. Rat blood was

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† Abbreviations: MIF-1, melanocyte stimulating hormone release inhibiting factor number 1; Tyr-MIF-1, tyrosine melanocyte stimulating hormone release inhibiting factor number 1; PCMB, *p*-chloromercuribenzoic acid; PTS-1, peptide transport system number 1; PKA, protein kinase A; TFA, trifluoroacetic acid.

obtained with a heparinized syringe from the aorta of adult white albino male rats (weighing about 300 g) purchased from Harlan Sprague Dawley (Indianapolis, IN) and anesthetized with sodium pentobarbital (65 mg/kg). Human blood was obtained with a heparinized syringe from the antecubital vein of healthy adults 22- to 30-years old. After centrifugation at 4°, the plasma was used immediately.

To 0.3 mL of fresh plasma was added about 1.7×10^6 dpm of tritiated peptide for duplicate measurements at each time point. Incubations were performed in a metabolic incubator (Dubnoff Incu-Shaker, Labline Instruments, Melrose Park, IL). Immediately, and at various specified times afterwards, samples were removed and placed in 10% TFA at 0° and centrifuged at 4°. The supernatants were dried in a Speed Vac (Savant Instruments, Farmingdale, NY), and the samples were stored in a dessicator at −20°.

Enzyme inhibitors were purchased from Sigma (St. Louis, MO). They were used at a final concentration of 3 μg/mL for bestatin, 1 mM for EDTA, 10 μM for PCMB, 1 μM for pepstatin, and 14.6 μL/mL (9 trypsin inhibiting units/mL) for aprotinin (Trasylol). The final volume of each sample was the same as in the samples tested without enzyme inhibitors. For the mixture, EDTA, PCMB, pepstatin, and aprotinin were used at a final concentration identical to that of each of the individual inhibitors.

For chromatography, the samples were reconstituted in solvent A (0.1% TFA in water), filtered (0.45 μm nylon 66 centrifuge filter, Alltech Associates, Deerfield, IL) and applied to a Brownlee RP-18 reversed phase column (4.6 mm × 22 cm) with a 3-cm RP 18 guard cartridge (Rainin, Woburn, MA). The HPLC system used was Beckman (Fullerton, CA) model 344 gradient with version 5.10 System Gold software. Solvent B was 0.1% TFA in methanol. The gradient for Tyr-MIF-1 consisted of 10% B that was increased gradually to 35% B over 40 min, maintained at 35% for an additional 20 min, and then increased to 80% B for 10 min. The gradient for MIF-1 was similar except that after 40 min, the 35% B was maintained for 5 min and was then increased to 80% for 20 min. The flow rate was 1 mL/min. Fractions of 1 mL were collected and counted in a liquid scintillation counter (Beckman model LS 3801). UV monitoring at 220 nm was used to define the standard retention times for Tyr-MIF-1, MIF-1 and their fragments before the biological samples were run. Tyr eluted at 11 min, Tyr-Pro at 21 min, Tyr-MIF-1 at 38 min, the free acid of Tyr-MIF-1 at 43 min, Tyr-Pro-Leu at 49 min, Pro at 5 min, Pro-Leu at 32 min, Leu-Gly-NH_2 at 29.5 min, the free acid of MIF-1 at 26 min, and MIF-1 at 22 min with the applied gradient. Before

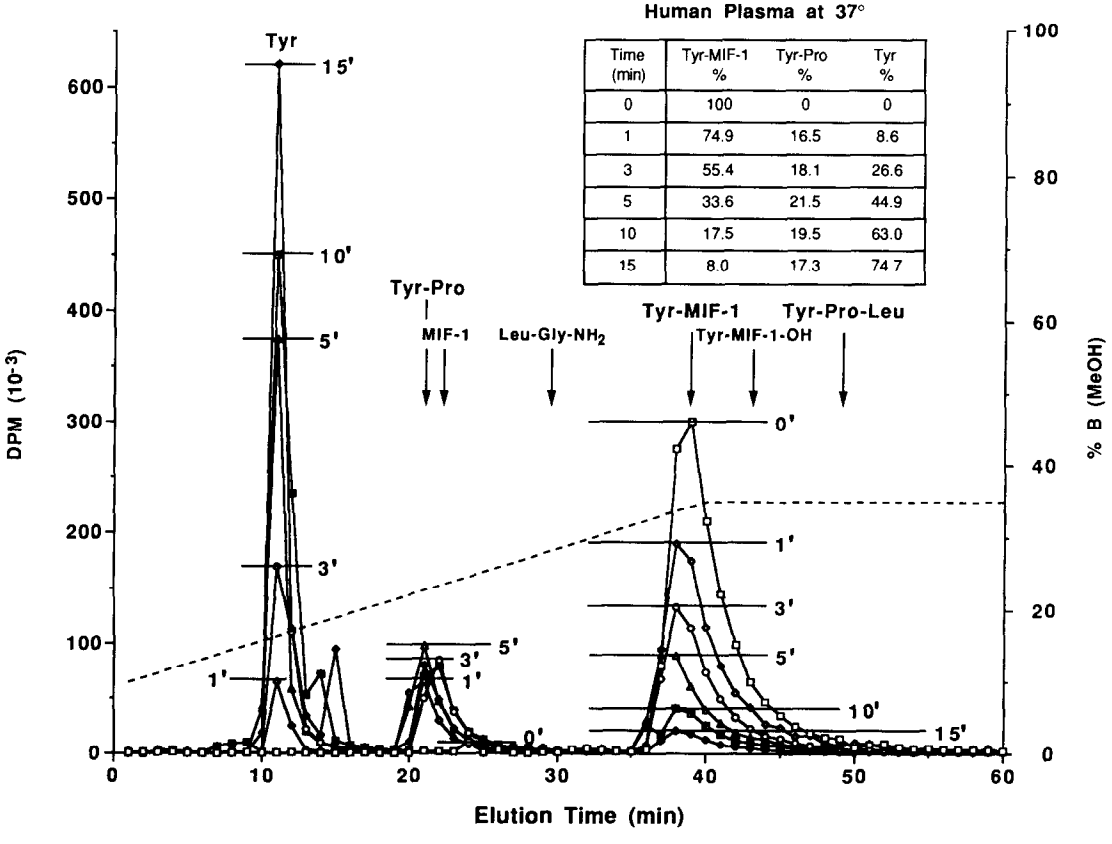
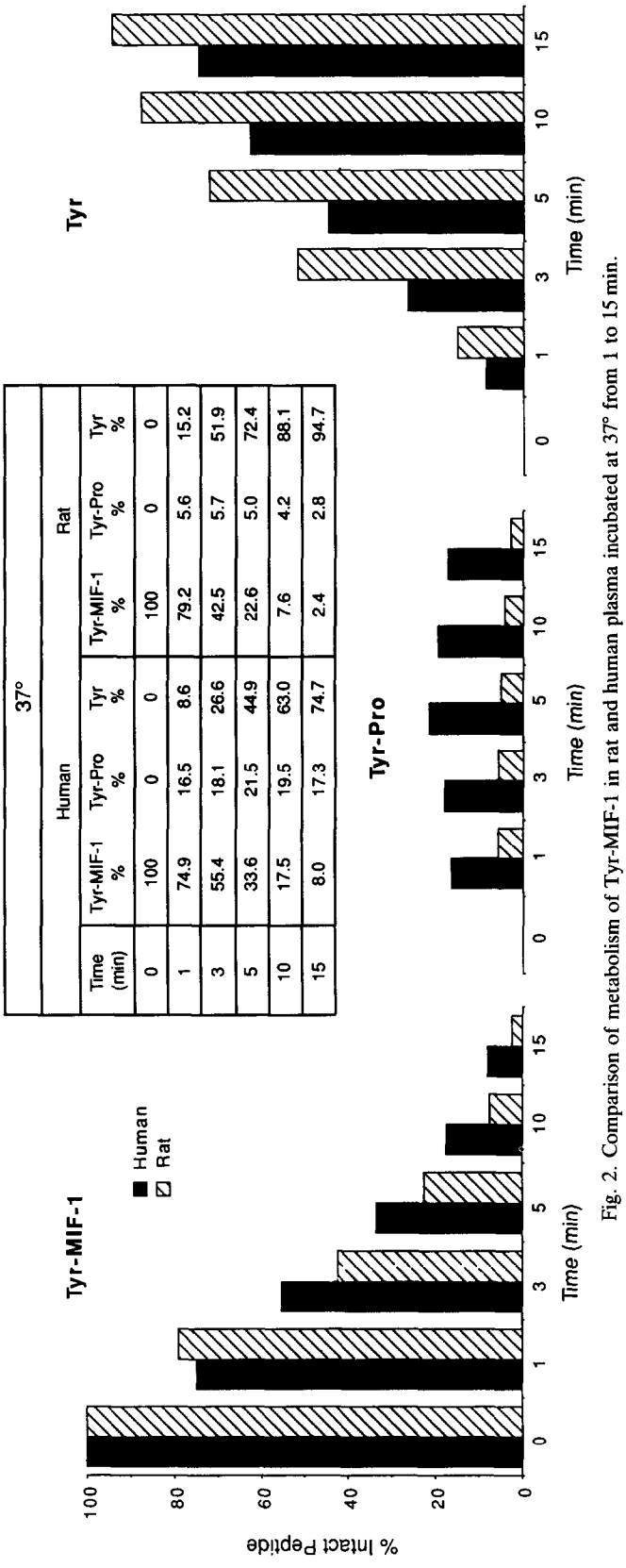


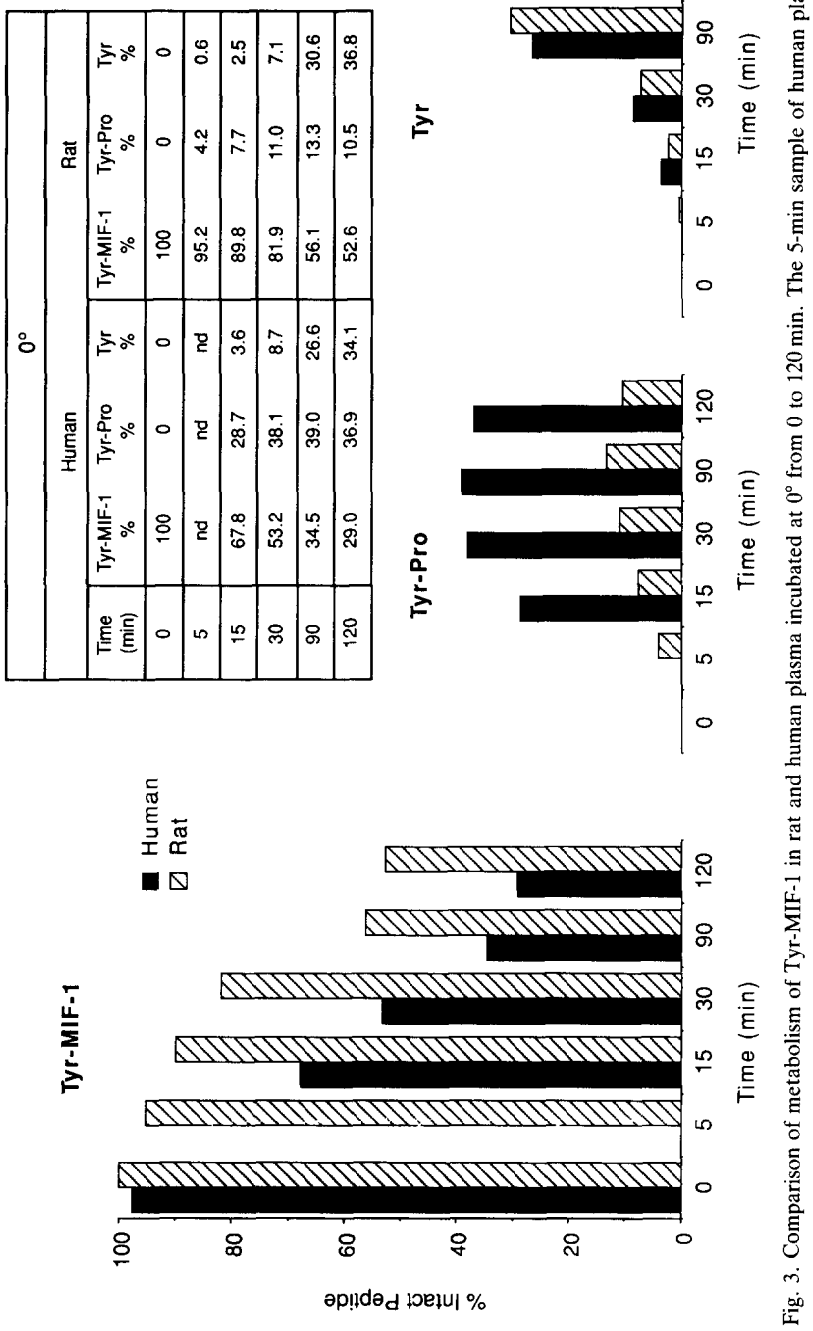
Fig. 1. HPLC chromatogram of tritiated Tyr-MIF-1 incubated in human plasma at 37°. The inset shows the percent of the major peaks of radioactivity. The dashed line represents the gradient (% B).



37°

	Human				Rat			
Time (min)	Tyr-MIF-1 %	Tyr-Pro %	Tyr %		Tyr-MIF-1 %	Tyr-Pro %	Tyr %	
0	100	0	0		100	0	0	
1	74.9	16.5	8.6		79.2	5.6	15.2	
3	55.4	18.1	26.6		42.5	5.7	51.9	
5	33.6	21.5	44.9		22.6	5.0	72.4	
10	17.5	19.5	63.0		7.6	4.2	88.1	
15	8.0	17.3	74.7		2.4	2.8	94.7	

Fig. 2. Comparison of metabolism of Tyr-MIF-1 in rat and human plasma incubated at 37° from 1 to 15 min.



0°								
	Human				Rat			
Time (min)	Tyr-MIF-1 %	Tyr-Pro %	Tyr %		Tyr-MIF-1 %	Tyr-Pro %	Tyr %	
0	100	0	0		100	0	0	
5	nd	nd	nd		95.2	4.2	0.6	
15	67.8	28.7	3.6		89.8	7.7	2.5	
30	53.2	38.1	8.7		81.9	11.0	7.1	
90	34.5	39.0	26.6		56.1	13.3	30.6	
120	29.0	36.9	34.1		52.6	10.5	36.8	

Fig. 3. Comparison of metabolism of Tyr-MIF-1 in rat and human plasma incubated at 0° from 0 to 120 min. The 5-min sample of human plasma was lost.

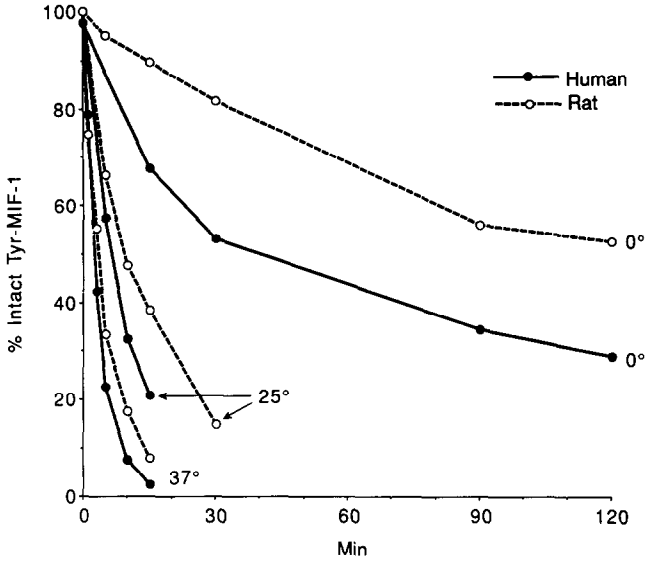


Fig. 4. Percent of Tyr-MIF-1 remaining in intact form after incubation in rat and human plasma at 0, 25, and 37°.

each analysis, the column was washed with 80% B for about 2 hr with the same gradient used for peptide and a blank sample was analyzed as a control. The fraction size was always 1 mL.

For *in vivo* studies, adult male albino rats weighing about 225 g were anesthetized with sodium pentobarbital (65 mg/kg), and about 1.0×10^8 dpm of Tyr-MIF-1 or MIF-1 was injected intravenously. Body temperature was monitored and maintained constant. Heparinized blood samples were obtained from the abdominal aorta and replaced with an equal volume of 0.9% NaCl. The samples were centrifuged at 1700 *g* for 3 min and the plasma was mixed with 10% TFA. The resulting dpm/mL were corrected for the hematocrit of each sample and for the percent of intact peptide determined by HPLC.

RESULTS

Figure 1 shows the chromatogram obtained by HPLC after tritiated Tyr-MIF-1 was incubated in human plasma for 0, 1, 3, 5, 10 and 15 min at 37°. Degradation of Tyr-MIF-1 resulted in the formation of Tyr-Pro and especially Tyr. No Tyr-Pro-Leu was detected. Similar findings were observed after the incubation of tritiated Tyr-MIF-1 in rat plasma at the same temperature. Incubation of the tritiated Tyr-MIF-1 with saline for 30 min at 37° revealed only a single peak without evidence of any degradation.

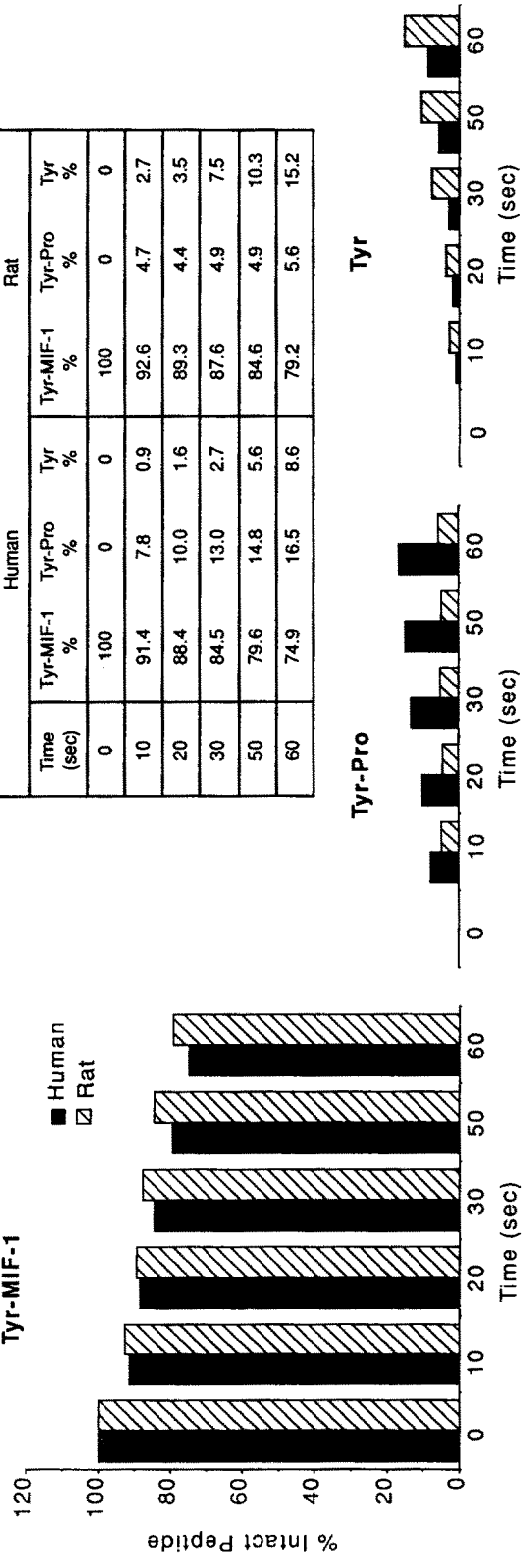
A direct comparison of the percentages of Tyr-MIF-1, Tyr, and Tyr-Pro measured after incubation of labeled Tyr-MIF-1 in human and rat plasma at 37° is shown in Fig. 2. Although significantly ($P < 0.0001$) more Tyr-Pro and less Tyr was formed after incubation in human plasma than in rat plasma at 37° and 25°, it was difficult to determine the relationship between the formation of Tyr and Tyr-Pro. At 0°, however, the formation of Tyr-Pro was more evident (Fig. 3).

Figure 4 shows the rates of metabolism of Tyr-MIF-1 at each of these temperatures on the same graph. The effect of temperature was highly significant ($P < 0.0001$) for both rat and human plasma. The half-time disappearance of Tyr-MIF-1 after incubation in rat plasma at 37° was 4.2 min, at 25° was 11.1 min, and at 0° was 125.0 min. In human plasma, the values were 2.8 min at 37°, 6.6 min at 25°, and 74.7 min at 0°. The degradation of Tyr-MIF-1 was about 62% faster in human than in rat plasma.

The results at 0° prompted us to look at shorter times of incubation of Tyr-MIF-1 to follow the relative formation of Tyr and Tyr-Pro. At times of 1 min or less in rat plasma (Fig. 5), significantly ($P < 0.001$) more Tyr-Pro than Tyr was formed after 10 sec of incubation, but this amount (about 5%) remained relatively constant for the next 10 min (Fig. 2), in contrast to the gradually increasing amounts of Tyr being formed. In human plasma, much more ($P < 0.0001$) Tyr-Pro than Tyr was formed during the first minute of incubation (Fig. 5), and the formation of Tyr did not exceed that of the dipeptide until 3 min (Fig. 2). Since MIF-1 does not contain Tyr, its formation could not be determined with the use of Tyr-MIF-1 labeled on the Tyr.

For this reason, Tyr-MIF-1 selectively tritiated on the Pro was prepared and incubated in rat or human blood at 37°. Samples were obtained as early as 30 sec, but no MIF-1 was detected. Even in the 3- and 5-min samples, in which the radioactivity eluting at the position of Pro represented most of the counts, no MIF-1 was found.

When the Tyr-MIF-1 tritiated on the Pro was mixed with the same amount of Tyr-MIF-1 tritiated on the Tyr and incubated in rat plasma at 37° for 5 min, almost identical amounts of tritiated Pro and tritiated Tyr were generated (Fig. 6). Addition of excess (1 mM) unlabeled Tyr-Pro to this incubation



0°							
	Human				Rat		
Time (sec)	Tyr-MIF-1 %	Tyr-Pro %	Tyr %		Tyr-MIF-1 %	Tyr-Pro %	Tyr %
0	100	0	0		100	0	0
10	91.4	7.8	0.9		92.6	4.7	2.7
20	88.4	10.0	1.6		89.3	4.4	3.5
30	84.5	13.0	2.7		87.6	4.9	7.5
50	79.6	14.8	5.6		84.6	4.9	10.3
60	74.9	16.5	8.6		79.2	5.6	15.2

Fig. 5. Comparison of metabolism of Tyr-MIF-1 in rat and human plasma incubated at 0° for times of 1 min or less

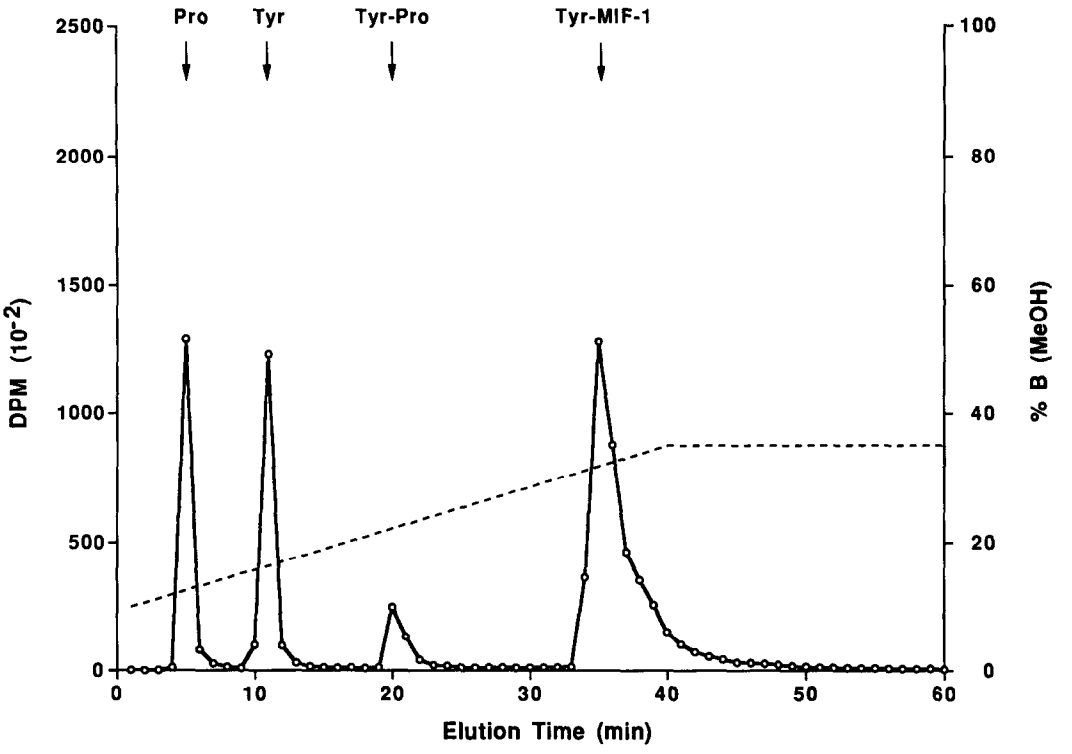


Fig. 6. HPLC chromatogram of a mixture of Tyr-MIF-1 tritiated at the Tyr and Tyr-MIF-1 tritiated at the Pro after incubation for 5 min in rat plasma at 37°. The dashed line represents the gradient (% B).

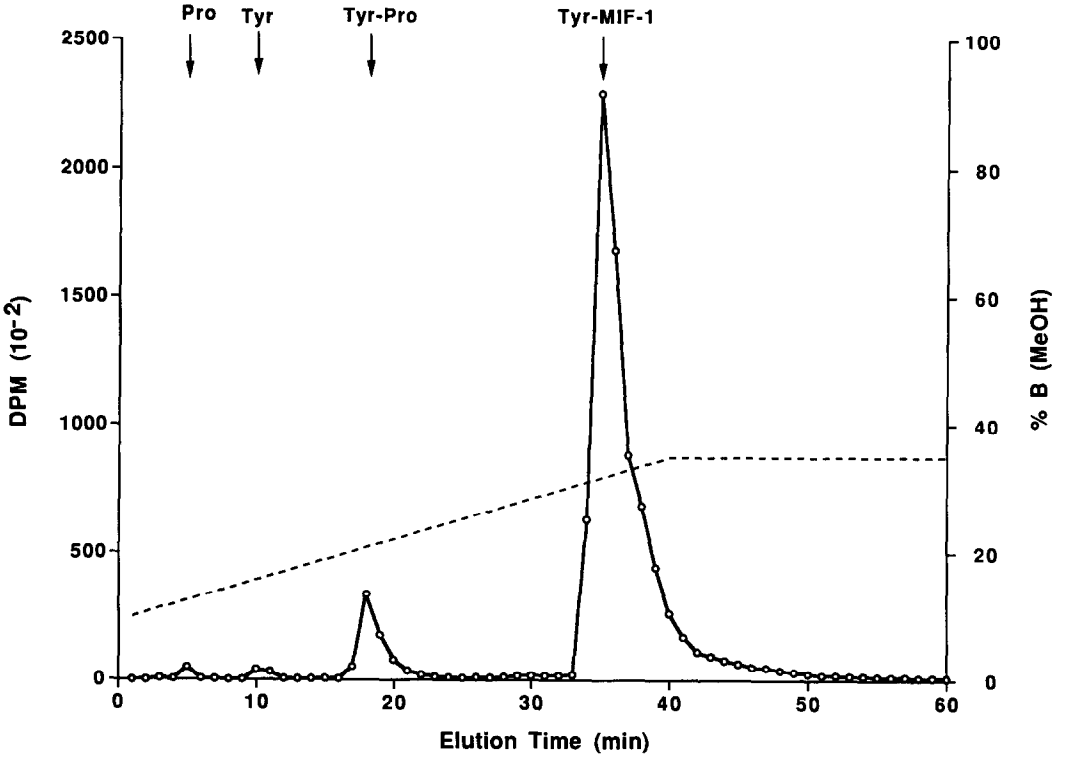


Fig. 7. HPLC chromatogram of the mixture of the two tritiated Tyr-MIF-1 peptides incubated under the same conditions as in Fig. 6 (rat plasma for 5 min at 37°) after addition of excess (1 mM) unlabeled Tyr-Pro. The dashed line represents the gradient (% B).

Table 1. Percent of Tyr-MIF-1 remaining in intact form after incubation in rat plasma at 37° with enzyme inhibitors

	Tyr-MIF-1 (%)	Tyr (%)
Control	32.5	61.6
Bestatin	29.4	61.3
EDTA	50.6	40.2
PCMB	30.5	60.7
Pepstatin	17.6	75.6
Aprotinin	40.4	50.2
Mixture	54.2	40.2

mixture almost completely and equally suppressed the formation of Tyr and Pro (Fig. 7). Similarly equal, but less, suppression was seen with 10 μM Tyr-Pro.

Several enzyme inhibitors were added to rat plasma incubated at 37°. At 10 min, EDTA and the mixture containing EDTA (in addition to pepstatin, aprotinin, and PCMB) were the most effective, but, as can be seen in Table 1, PCMB, bestatin, and pepstatin were without beneficial effect. At 30 min, less than a third of the Tyr-MIF-1 remained intact with any enzyme inhibitor, but bestatin seemed to be more effective than EDTA at this later time. When all the times were combined, the mixture did the best overall, resulting in a half-time disappearance of Tyr-MIF-1 that was about twice as long as seen without the inhibitors.

The tritiated tripeptide MIF-1 also was incubated in rat and human plasma at 37° and these chromatograms are shown in Figs. 8 and 9. In rat plasma, less than half the MIF-1 remained intact after incubation for 30 min in contrast to more than half the MIF-1 remaining intact after incubation for 5 days in human plasma. In Fig. 10, the results of incubation of MIF-1 and Tyr-MIF-1 in human and rat plasma are shown together in a different format. The markedly delayed disappearance of MIF-1 in human plasma is evident even during the first hour of incubation. At 5 days, 50.9% of MIF-1 incubated in human plasma at 37° remained intact. When calculated as the log% intact MIF-1 remaining over time, the half-time disappearance becomes 8.8 days (4.8 days for the first 7 points) as compared with a time of 35.7 min calculated in the same way for this peptide incubated in rat plasma.

The *in vivo* studies in the rat showed a rapid disappearance from blood of both Tyr-MIF-1 and MIF-1. As in the *in vitro* studies, the disappearance of Tyr-MIF-1 was quicker than that of MIF-1. If a semilogarithmic relationship is assumed for the disappearance curve for Tyr-MIF-1, the half-time disappearance is 0.46 min with a volume of distribution of 83.7 mL or 37.2% body weight.

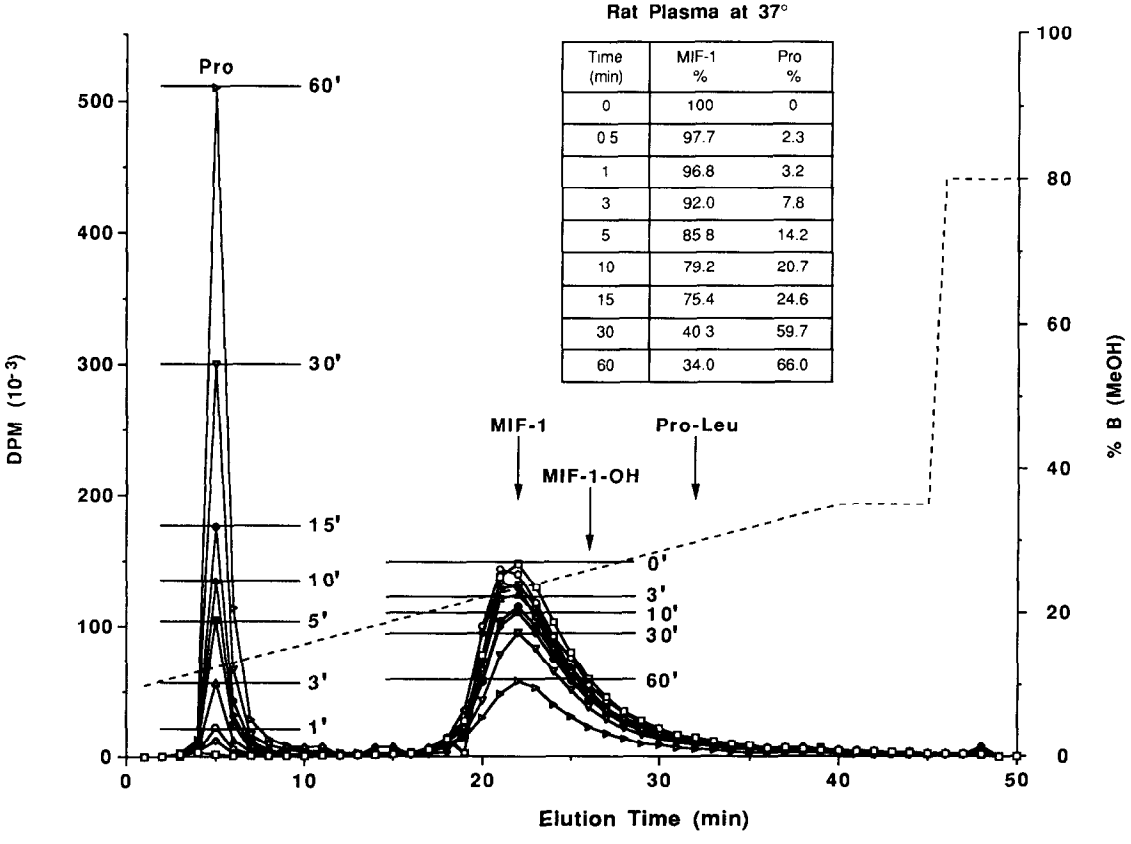


Fig. 8. HPLC chromatogram of tritiated MIF-1 incubated in rat plasma at 37°. The inset shows the percent of the major peaks of radioactivity. The dashed line represents the gradient (% B).

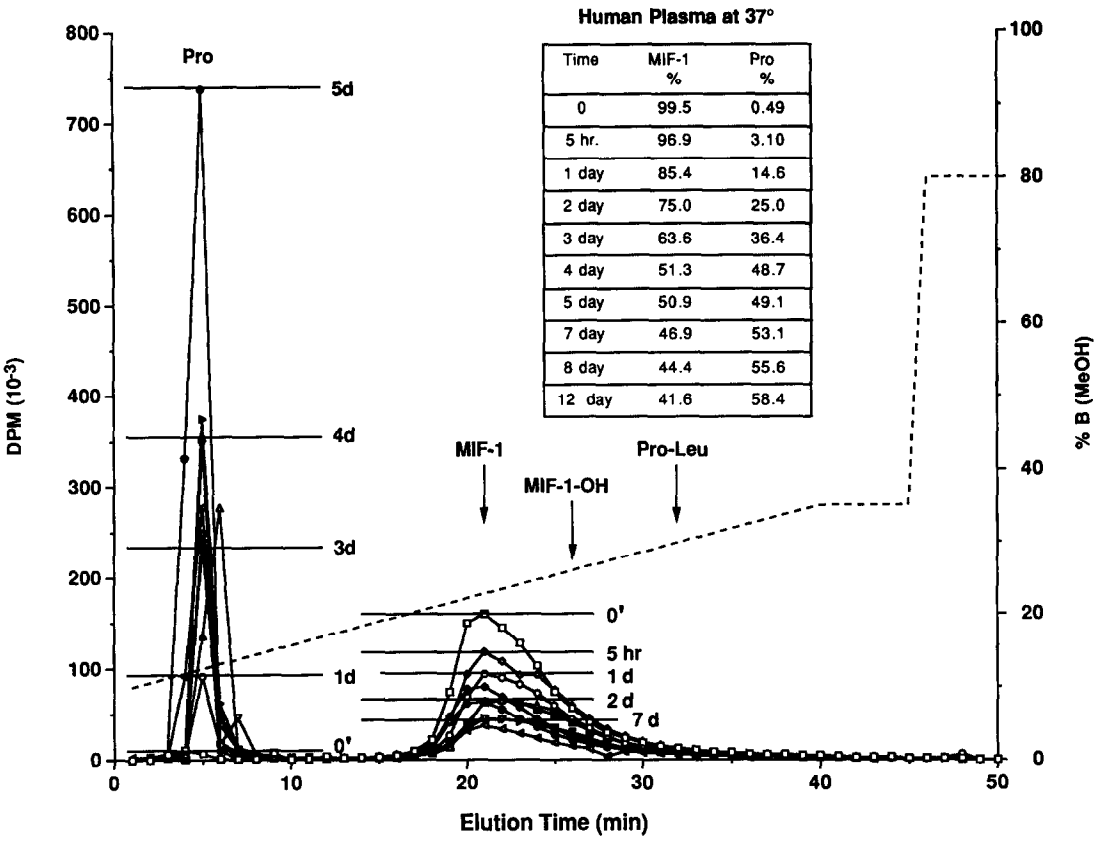


Fig. 9. HPLC chromatogram of tritiated MIF-1 incubated in human plasma at 37°. The inset shows the percent of the major peaks of radioactivity. The dashed line represents the gradient (% B).

For MIF-1, a biphasic relationship best fits the determinations. The half-time disappearance of MIF-1 for the first phase probably represents equilibration and is 0.07 min; for the second phase, the half-time disappearance is 12.2 min. The volume of distribution for the first phase is 0.7 mL and for the second phase is 45.9 mL, representing 20.4% of body weight.

DISCUSSION

The results show that Tyr-MIF-1 was metabolized rapidly in both rat and human plasma. Within 5 min, more than half the intact tetrapeptide had been degraded at 37°. Even at 0°, only slightly more than half of the material remained intact in human plasma at 30 min. The results in human plasma for the

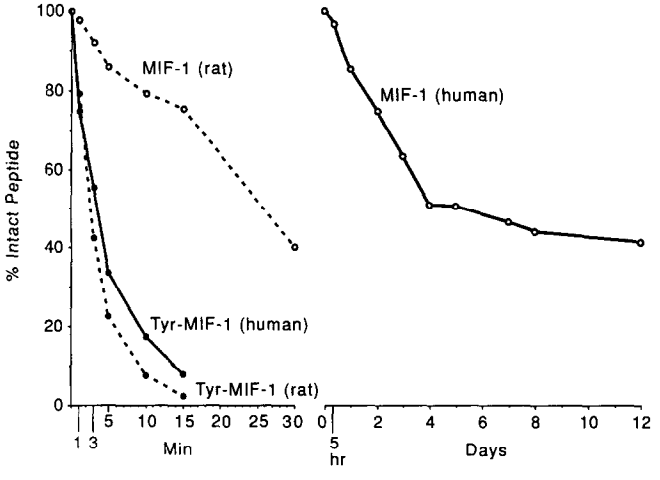


Fig. 10. Percent of MIF-1 and Tyr-MIF-1 remaining in intact form after incubation in rat and human plasma at 37°.

tetrapeptide Tyr-MIF-1 were in marked contrast to those for the tripeptide MIF-1.

For MIF-1, incubation in human plasma at 37° showed that more than half the peptide remained intact at five days. These results are consistent with those previously reported *in vivo* [22] and *in vitro* [23, 24]. In rat plasma, although the metabolism of MIF-1 was much more rapid than in human plasma, it still was slower than that of Tyr-MIF-1, particularly after the first 5 min.

Each experiment involved identification of all labeled products at each time by HPLC. At 37°, the formation of Tyr-Pro was less than the formation of Tyr. It was only after the metabolism of Tyr-MIF-1 was slowed by incubation at 0° that the formation of Tyr-Pro became more evident, particularly in human plasma. This was substantiated by incubation of Tyr-MIF-1 at shorter times at 37°.

Additional evidence against Tyr-MIF-1 functioning as a major precursor of MIF-1 was provided by the subsequent synthesis and incubation in plasma of Tyr-MIF-1 labeled with tritium on the Pro (rather than on the Tyr). In neither rat nor human plasma could the formation of MIF-1 be detected. The generation of almost identical amounts of tritiated Pro and tritiated Tyr after both tritiated tetrapeptides were mixed and incubated with rat plasma is consistent with the predominant generation of Tyr-Pro followed by degradation to Tyr and Pro rather than with the initial formation of Tyr and MIF-1. Further support for this pathway was provided by the addition of excess (1 mM) unlabeled Tyr-Pro to the incubation mixture of the two tritiated tetrapeptides; this almost completely suppressed the formation of both Tyr and Pro. The evidence indicates, therefore, that the predominant metabolism of Tyr-MIF-1 in blood is to Tyr-Pro rather than to MIF-1.

Although relatively more Tyr-Pro than Tyr was formed after incubation of Tyr-MIF-1 in human plasma than in rat plasma, the metabolism of Tyr-MIF-1 in plasma from both species was rapid. The rate of metabolism of MIF-1 in rat plasma was also relatively rapid, as would be expected of a small peptide. The markedly prolonged degradation of MIF-1 in human plasma, however, raises the possibility of different or more prolonged actions of this tripeptide in the human being.

In vivo, the half-time disappearance of intact [^{3}H]MIF-1 after intravenous injection in the rat was 12.2 min, a value in relative agreement with that found in an earlier study in which a different anesthetic was used [21]. The half-time disappearance of Tyr-MIF-1 *in vivo* has not been reported before but, as expected from our *in vitro* results, was more rapid than that of MIF-1. For both Tyr-MIF-1 and MIF-1, the volumes of distribution were much greater than plasma volume. Even if peptide entered red blood cells, for which there is evidence [28], the volume of distribution for Tyr-MIF-1 and MIF-1 would still be at least double that of the entire vascular space.

Thus, the metabolism of Tyr-MIF-1 in blood is faster than that of MIF-1, particularly in human plasma where MIF-1 is remarkably stable, and results in the predominant formation of Tyr-Pro rather than MIF-1.

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REFERENCES

1. Nair RMG, Kastin AJ and Schally AV, Isolation and structure of hypothalamic MSH release-inhibiting hormone. *Biochem Biophys Res Commun* **43**: 1376–1425, 1971.
2. Horvath A and Kastin AJ, Isolation of tyrosine-melanocyte-stimulating hormone release-inhibiting factor 1 from bovine brain tissue. *J Biol Chem* **264**: 2175–2179, 1989.
3. Horvath A and Kastin AJ, Evidence for presence of Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) in human brain cortex. *Int J Pept Protein Res* **36**: 281–284, 1990.
4. Kastin AJ, Abel DA, Ehrensing RH, Coy DH and Graf MV, Tyr-MIF-1 and MIF-1 are active in the water wheel test for antidepressant drugs. *Pharmacol Biochem Behav* **21**: 767–771, 1984.
5. Niesink RJM and van Ree JM, Neuropeptides and social behavior of rats tested in dyadic encounters. *Neuropeptides* **4**: 483–496, 1984.
6. Galina ZH and Kastin AJ, Existence of antiopiate systems as illustrated by MIF-1/Tyr-MIF-1. Minireview. *Life Sci* **39**: 2153–2159, 1986.
7. Kavaliers M and Hirst M, Inhibitory influences of MIF-1 (PLG) and Tyr-MIF-1 (YPLG) on aggression and defeat-induced analgesia in mice. *Peptides* **7**: 1007–1010, 1986.
8. Kavaliers M, MIF-1 and Tyr-MIF-1 antagonize morphine and opioid but not non-opioid stress-induced analgesia in the snail, *Cepaea nemoralis*. *Peptides* **8**: 1–5, 1987.
9. Pulvirenti L and Kastin AJ, Blockade of brain dopamine receptors antagonizes the anti-immunobility effect of MIF-1 and Tyr-MIF-1 in rats. *Eur J Pharmacol* **151**: 289–292, 1988.
10. Lipa SM, Kavaliers M and Ossenkopp K-P, Differential inhibitory effects of MIF-1, Tyr-MIF-1, naloxone and β-funaltrexamine on body rotation-induced analgesia in the meadow vole, *Microtus pennsylvanicus*. *Peptides* **10**: 493–497, 1989.
11. Kostowski W, Danysz W, Dyr W, Jankowska E, Krzascik P, Palejko W, Stefanski R and Plaznik A, MIF-1 potentiates the action of tricyclic antidepressants in an animal model of depression. *Peptides* **12**: 915–918, 1991.
12. Ehrensing RH and Kastin AJ, Dose-related biphasic effect of prolyl-leucyl-glycinamide (MIF-1) in depression. *Am J Psychiatry* **135**: 562–566, 1978.
13. van der Velde CD, Rapid clinical effectiveness of MIF-1 in the treatment of major depressive illness. *Peptides* **4**: 297–300, 1983.
14. Ehrensing RH, Kastin AJ and Michell GF, Antagonism of morphine analgesia by prolyl-leucyl-glycinamide (MIF-1) in humans. *Pharmacol Biochem Behav* **21**: 975–978, 1984.
15. Luciano MG, Zadina JE, Kastin AJ and Coy DH, Mu and delta opiate receptors in rat brain are affected by GTP but not MIF-1. *Brain Res Bull* **7**: 677–682, 1981.
16. Zadina JE and Kastin AJ, Interactions of Tyr-MIF-1 at opiate receptor sites. *Pharmacol Biochem Behav* **25**: 1303–1305, 1986.
17. Banks WA and Kastin AJ, A brain-to-blood carrier-mediated transport system for small, N-tyrosinated

peptides. *Pharmacol Biochem Behav* **21**: 943–946, 1984.
18. Banks WA, Kastin AJ, Fischman AJ, Coy DH and Strauss SL, Carrier-mediated transport of enkephalins and N-Tyr-MIF-1 across blood–brain barrier. *Am J Physiol* **251**: E477–E482, 1986.
19. Zadina JE, Kastin AJ, Kersh D and Wyatt A, Tyr-MIF-1 can act as an opiate agonist as well as antagonist in the guinea pig ileum. *Life Sci* **51**: 869–885, 1992.
20. Zhang X-Y, Zadina JE, Ehrlich M and Kastin AJ, Tyr-MIF-1, but not MIF-1 or morphine, decreases cyclic AMP-dependent protein kinase activity extracted from HeLa cells. *Mol Cell Neurosci* **3**: 348–353, 1992.
21. Redding TW, Kastin AJ, Nair RMG and Schally AV, Distribution, half-life, and excretion of ^{14}C- and ^{3}H-labeled L-prolyl-L-leucyl-glycinamide in the rat. *Neuroendocrinology* **11**: 92–100, 1973.
22. Redding TW, Kastin AJ, Gonzalez-Barcena D, Coy DH, Hirotsu Y, Ruelas J and Schally AV, The disappearance, excretion, and metabolism of tritiated prolyl-leucyl-glycinamide in man. *Neuroendocrinology* **16**: 119–126, 1974.
23. Walter R, Neidle A and Marks N, Significant differences in the degradation of Pro-Leu-Gly-NH_2 by human serum and that of other species. *Proc Soc Exp Biol Med* **148**: 98–103, 1975.
24. Witter A, Scholtens H and Verhoef J, H-Pro-[^{3}H]Leu-Gly-NH_2: Metabolism in human and rat plasma investigated by high-pressure liquid chromatography. *Neuroendocrinology* **30**: 377–381, 1980.
25. Walter R, Griffiths EC and Hooper KC, Production of MSH-release-inhibiting hormone by a particulate preparation of hypothalami: Mechanisms of oxytocin inactivation. *Brain Res* **60**: 449–457, 1973.
26. Burbach JPH, De Kloet ER and De Wield D, Oxytocin biotransformation in the rat limbic brain: Characterization of peptidase activities and significance in the formation of oxytocin fragments. *Brain Res* **202**: 401–414, 1980.
27. Marks N, Berg MJ, Kastin AJ and Coy DH, Evidence for conversion of N-Tyr-MIF-1 into MIF-1 by a specific brain aminopeptidase. *Neurochem Int* **6**: 347–353, 1984.
28. Banks WA and Kastin AJ, Uptake of peptides containing Tyr-Pro by human and mouse erythrocytes. *Biochem Pharmacol* **40**: 607–614, 1990.