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Regional Differences in the Metabolism of Tyr-MIF-1 and Tyr-W-MIF-1 by Rat Brain Mitochondria

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ABSTRACT. Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) and Tyr-W-MIF-1 (Tyr-Pro-Trp-Gly-NH_2) are endogenous neuropeptides with opiate modulating and other CNS effects. After incubation of the tritiated tetrapeptides with fractions of tissue from different areas of rat brain, formation of the metabolites was determined by HPLC. Marked regional differences in degradation were found for both peptides. The metabolism of Tyr-MIF-1, resulting in the formation of the biologically active MIF-1 (Pro-Leu-Gly-NH_2), was greater in the mitochondrial than in the synaptosomal fractions. In the mitochondrial fraction, about twice as much MIF-1 was formed in brain cortex than in striatum, diencephalon, or midbrain/pons medulla. These results, showing differential metabolism in various areas of the brain, indicate another means for regulation of the concentrations of neuropeptides. BIOCHEM PHARMACOL **55**;1:33–36, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. metabolism; Tyr-MIF-1; Tyr-W-MIF-1; MIF-1; brain; HPLC

Study of the metabolism of members of the Tyr-MIF-1† family of peptides has revealed several unexpected findings for these endogenous [1–6] neuropeptides. These include the marked resistance of MIF-1 to degradation in human blood [7], the delayed degradation of Tyr-MIF-1 in neonatal rat blood [8] as well as in adult rat cerebrospinal fluid [9], and the metabolism of Tyr-MIF-1 into MIF-1 by brain mitochondria but not by brain homogenates [10] or blood [7].

Tyr-W-MIF-1, like Tyr-MIF-1, has been isolated from human brain cortex [3, 6], but its metabolism has not been studied. Both neuropeptides can show opiate as well as antiopiate activity [3, 11] and are saturably transported across the blood–brain barrier (BBB) in the direction of brain to blood by the same system that transports Met-enkephalin [12–14].

By contrast, MIF-1 has antiopiate [15] but not opiate activity and is not transported out of the brain. Unlike Tyr-MIF-1 and Tyr-W-MIF-1, it is saturably transported into the brain and crosses the BBB in this direction at a rate faster than that of morphine [16]. The BBB, therefore, provides a regulatory function for members of the Tyr-MIF-1 family of peptides.

Another form of regulation could be exerted by differential enzymatic activity in various parts of the brain. This would provide a different way for modulation of the ratio of MIF-1 to Tyr-MIF-1, as well as for inactivation of Tyr-W-MIF-1 and Tyr-MIF-1. Since only the mitochondrial fraction of brain has been shown to generate MIF-1 from Tyr-MIF-1, we focused on the metabolism of Tyr-W-MIF-1 and Tyr-MIF-1 in this fraction from several regions of the rat brain.

MATERIALS AND METHODS

Tyr-MIF-1 and Tyr-W-MIF-1 were tritiated (Amersham, Arlington Heights, IL) on the Pro from the [dehydro-Pro]^2-precursors. Adult albino male rats obtained from Harlan Sprague–Dawley (Indianapolis, IN) were decapitated according to a procedure approved by the Animal Use Committee, and whole brain was removed, placed on a cold plate on ice, and quickly dissected into component regions. Each region was homogenized immediately in 10 vol. of cold 0.32 M sucrose with a Teflon pestle in a Potter–Elvehjem apparatus stirred by a motor at a rotational speed for the shaft of 800 rpm (6 complete up-and-down strokes), a process repeated after cooling in ice for 30 sec. Subsequent steps were performed on ice.

The homogenate was centrifuged for 10 min at 1000 × *g*, and the pellet was resuspended and centrifuged again. The supernatants were combined and centrifuged at 17,500 × *g* for 20 min. The resulting supernatant was centrifuged at 30,000 × *g* for 20 min to yield the supernatant S_2 and a pellet that was added to the pellet from the previous centrifugation to form the combined pellet P_2. This pellet was homogenized by hand in 0.32 M sucrose (2–3 mL/g of brain tissue), layered on a step density gradient (0.32, 0.8, and 1.2 M sucrose) prepared 1 hr earlier, and centrifuged for 2 hr at 50,000 × g. The resulting pellet constituted the mitochondrial fraction, which was homogenized by hand in

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† *Abbreviations:* Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2), tyrosine-MIF-1; MIF-1 (Pro-Leu-Gly-NH_2), melanocyte-stimulating hormone release-inhibiting factor-1; Tyr-W-MIF-1 (Tyr-Pro-Trp-Gly-NH_2); TFA, trifluoroacetic acid; and spm, synaptosomal plasma membrane.

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0.02 M Tris buffer (pH 7.6) immediately before incubation with the tritiated tetrapeptides.

The spm fraction was prepared from the synaptosomal layer between 0.8 and 1.2 M sucrose to which was added 11 vol. of 20 mM cold Tris–HCl (pH 7.6). This was stirred, left on ice for 1 hr, and centrifuged at 16,500 × *g* for 20 min. The resulting pellet constituted the spm fraction, which was homogenized by hand in 20 mM Tris–HCl immediately before incubation.

Immediately before incubation, the purity of the tritiated peptides was checked by HPLC; each was found to elute as a single, sharp peak. For the incubation, 3.5×10^6 dpm of each of the tritiated peptides was added to 0.3 mL of brain fractions preheated to 37° in a metabolic shaker (Dubnoff Incu-shaker; Labline Instruments, Melrose Park, IL). Samples were removed, placed in ice-cold 10% TFA for 30 min, and centrifuged at 4°. The supernatants were dried in a Speed Vac (Savant Instruments, Farmingdale, NY), and the samples were stored in a desiccator at −20° until chromatography.

For chromatography, the samples were reconstituted in solvent (0.1% TFA in water), filtered (nylon 66 centrifuge filter; pore size, 0.45 μm; Alltech Associates, Deerfield, IL), and applied to a Brownlee RP-18 reversed-phase column (4.6 mm × 22 cm) with a 1.5 cm RP-18 guard cartridge (Rainin, Woburn, MA). The HPLC system used was a Beckman (Fullerton, CA) model 344 with version 5.10 Gold software. Solvent B was 0.1% TFA in methanol. The gradient consisted of 10% solvent B gradually increased to 35% solvent B over 40 min, maintained at 35% for an additional 20 min, and then increased to 80% solvent B for 10 min.

For Tyr-MIF-1 with this gradient, Pro eluted at 5 min, MIF-1 at 17 min, Tyr-Pro at 21 min, MIF-1-OH at 26 min, Leu-Gly-NH_2 at 29.5 min, Pro-Leu at 32 min, Tyr-MIF-1 at 38 min, Tyr-MIF-1-OH at 43 min, and Tyr-Pro-Leu at 49 min. For Tyr-W-MIF-1, Pro-Trp-Gly-NH_2 eluted at 24 min, Pro-Trp-Gly-OH at 31 min, Tyr-W-MIF-1 at 50 min, Tyr-Pro-Trp-Gly-OH at 53 min, and Tyr-Pro-Trp-OH at 65 min. Before each analysis, the column was washed with 80% methanol for 2 hr, then twice with the same gradient used for the peptides. A blank sample was analyzed as a control. Samples were collected as 1-mL fractions, diluted to normalize for protein content, and counted for radioactivity by liquid scintillation.

The half-time disappearance rates of Tyr-MIF-1 and Tyr-W-MIF-1 incubated in mitochondrial fractions of brain were determined from the slope of the log (% intact peptide) versus time.

RESULTS

At each time examined, there was less Tyr-MIF-1 (Fig. 1) and Tyr-W-MIF-1 (Fig. 2) remaining intact in the mitochondrial fraction from brain cortex than from striatum, diencephalon, or cerebellum. For Tyr-MIF-1, there was an inverse relationship with the formation of the MIF-1 tripeptide such that the greater the metabolism of Tyr-MIF-1, the greater the formation of MIF-1. This was particularly evident in cortex (Fig. 3). For Tyr-W-MIF-1, relatively less of the Pro-Trp-Gly-NH_2 tripeptide was formed at each time in each tissue, even in cortex where the metabolism of Tyr-W-MIF-1 was the greatest (Fig. 4).

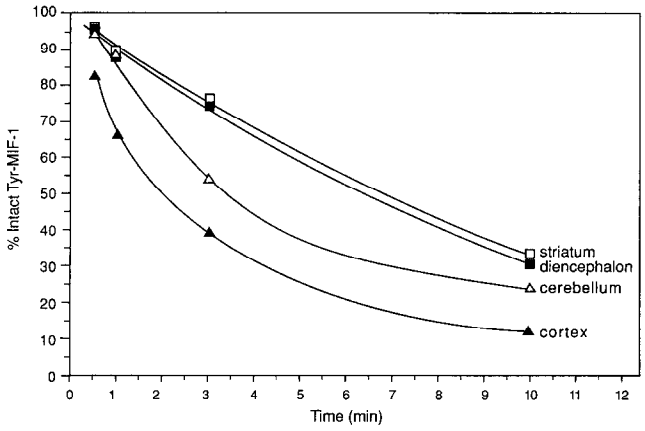

FIG. 1. Percent of Tyr-MIF-1 remaining in intact form, determined by HPLC, after incubation with the mitochondrial fraction from various regions of the brain at 37° for four different times. Starting amount of peptide at each time point was 3.5×10^6 dpm.

The formation of tripeptide from Tyr-MIF-1 (Fig. 3) and, to a lesser extent, from Tyr-W-MIF-1 (Fig. 4) was much greater in the mitochondrial fractions of each region of the brain than in the spm fractions. In contrast to incubations with mitochondria, preliminary studies indicated that there did not seem to be any loss of enzymatic activity when the incubation of peptide was performed on spm kept overnight on ice.

A replication of the study of the metabolism of Tyr-MIF-1 at 3 min in rats agreed well with the first experiment. As was found initially, the highest percent of radioactivity from the added Tyr-MIF-1 that eluted at the position of

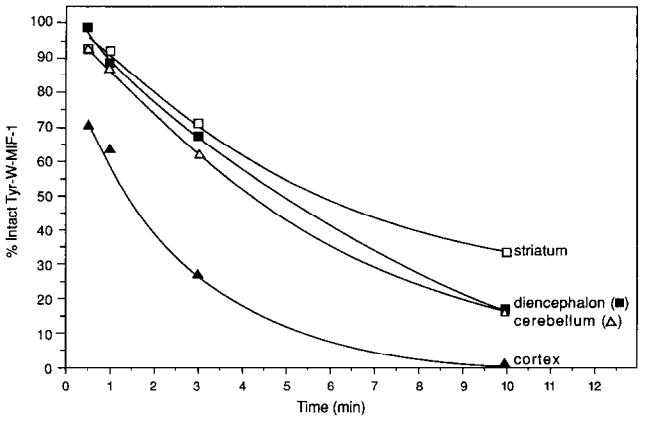

FIG. 2. Percent of Tyr-W-MIF-1 remaining in intact form, determined by HPLC, after incubation with the mitochondrial fraction from various regions of the brain at 37° for four different times. Starting amount of peptide at each time point was 3.5×10^6 dpm.

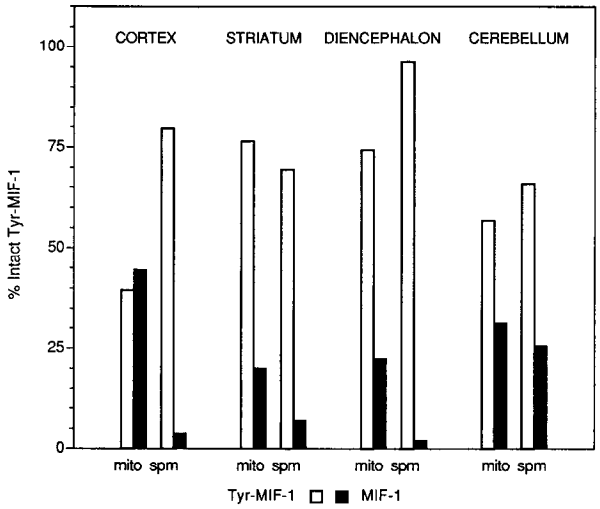

FIG. 3. Percent of radioactivity eluting as the C-terminal tripeptide MIF-1, determined by HPLC, after incubation of tritiated Tyr-MIF-1 for 3 min at 37° in fractions of brain mitochondria (mito) and synaptosomal plasma membrane (spm). Starting amount of peptide was 3.5 × 10^6 dpm.

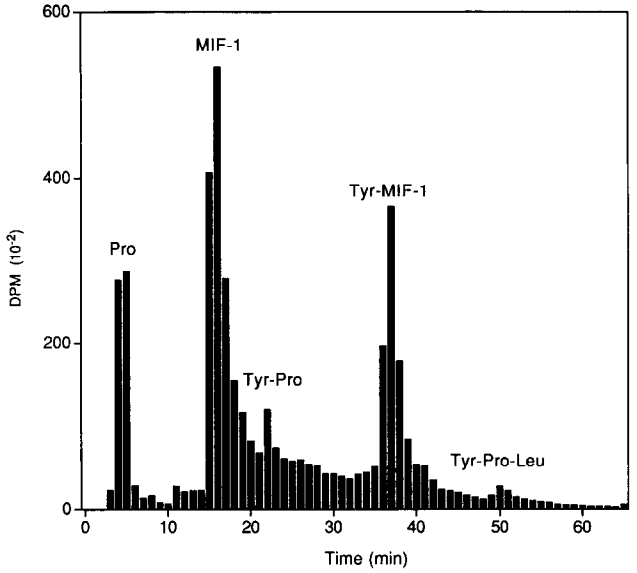

FIG. 5. HPLC chromatogram of tritiated Tyr-MIF-1 after incubation for 3 min at 37° in the mitochondrial fraction of rat brain cortex.

MIF-1 (43.4 vs 44.5% in the main study) occurred in the cortex, as illustrated in Fig. 5. The lowest formation of MIF-1 again was found in the diencephalon (23.8 vs 22.5% in the main study) and in the midbrain/pons medulla (21.7%), a section not initially dissected.

A preliminary study of degradation of the tritiated tetrapeptides in brain cortex also was performed in mice at 3 min of incubation. The results indicated that generally there was less degradation at this time in mouse cortex than in rat cortex. Only about 20% of the radioactivity from the tritiated Tyr-MIF-1 added to mouse cortex eluted as MIF-1.

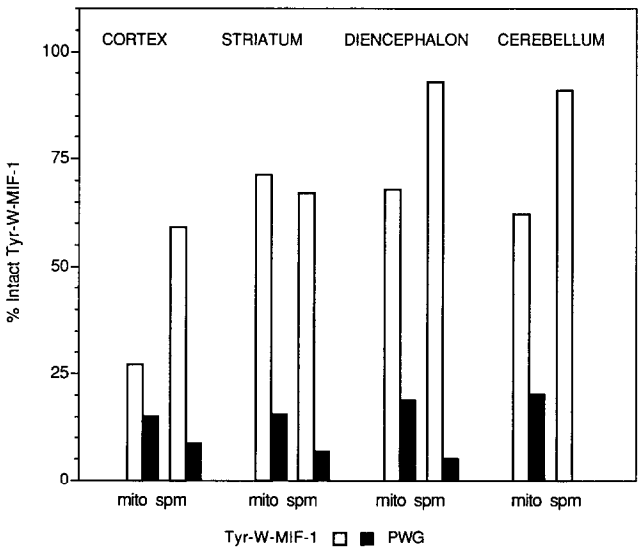

FIG. 4. Percent of radioactivity eluting as the C-terminal tripeptide Pro-Trp-Gly (PWG), determined by HPLC, after incubation of Tyr-W-MIF-1 for 3 min at 37° in fractions of brain mitochondria (mito) and synaptosomal plasma membrane (spm). Starting amount of peptide was 3.5 × 10^6 dpm.

Calculation of the half-time disappearance of Tyr-MIF-1 after incubation in the mitochondrial fractions showed a significant effect of the region of the brain from which the fractions were obtained: $F(6, 12) = 12.8$, $P < 0.001$. The half-life of Tyr-MIF-1 in the cortical fractions was 3.50 min, a time significantly ($P < 0.01$) faster than that in striatum, diencephalon, or cerebellum. The half-time disappearances from these other regions were not significantly different from each other and ranged from 4.95 to 6.31 min.

Similar calculations for Tyr-W-MIF-1 showed a significant effect of the region of the brain: $F(6, 11) = 138$, $P < 0.0001$. As with Tyr-MIF-1, the half-time disappearance (1.66 min) of Tyr-W-MIF-1 in the mitochondrial fractions of the cortex was significantly ($P < 0.001$) faster than that of the other three regions examined, none of which were significantly different from the other, with half-times ranging from 3.83 to 6.51 min. The longest half-life was in the striatum, followed by the diencephalon, cerebellum, and cortex. The same order was seen for Tyr-MIF-1. The half-life in the cortex was significantly ($P < 0.05$) faster for Tyr-W-MIF-1 than for Tyr-MIF-1.

DISCUSSION

The results show that at 3 min the metabolism of Tyr-MIF-1 was about twice as great in mitochondria from the cortex of the brain than from several other regions examined. This had the effect of generating more MIF-1 in the cortex than in the striatum, diencephalon, cerebellum, or midbrain/pons medulla.

Similarly, the metabolism of Tyr-W-MIF-1 at 3 min was about twice as great in cortical mitochondria than in other parts of the brain. By contrast with the formation of MIF-1 from Tyr-MIF-1, however, the comparable C-terminal tri-

peptide of Tyr-W-MIF-1 (Pro-Trp-Gly-NH_2) was not formed at any greater rate in the cortex than in any of the other brain parts examined. Yet, formation of both tripeptides involves a split of the Tyr-Pro bond.

In the spm fraction, degradation of Tyr-MIF-1 and Tyr-W-MIF-1 was similar in all brain parts. For Tyr-MIF-1 at 3 min in cortical spm, only about 4% of the total radioactivity eluted at the position of MIF-1 as compared with more than ten times that amount in the mitochondrial fraction of the cortex.

More MIF-1 was formed in the mitochondrial fraction of each brain part than in the spm fraction. This agrees with our previous results with tissue obtained from whole brain, in which the formation of MIF-1 from Tyr-MIF-1 was much greater in the mitochondrial fraction than in the microsomal fraction, spm fraction, P_2 fraction, or crude brain homogenate [10].

Thus, Tyr-MIF-1 and Tyr-W-MIF-1 are differentially metabolized in various parts of the brain. This is consistent with the variations in regional localization of cerebral peptidases reported in the mouse [17] and provides another mechanism by which the biological activity of peptides can be regulated.

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