Immunoreactive Plasma Concentrations of an Endogenous Antiopiate Are Higher in Spontaneously Hypertensive Rats Than in Wister-Kyoto Rats

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SUMMARY Tyrosine-MIF-1 (Tyr-Pro-Leu-Gly-NH₂) is present in rat brain in varying concentrations throughout the day and can act as an opiate antagonist. Since altered sensitivity to pain is known to occur in hypertension, plasma and brain concentrations of Tyr-MIF-1-like immunoreactivity were measured in spontaneously hypertensive rats (SHR) and compared every 4 hours for 24 hours with the concentrations in control Wistar-Kyoto rats (WKY). The Tyr-MIF-1-like immunoreactivity in plasma was significantly higher in SHR than in the WKY at each interval; the mean difference was 62% (p < 0.001). High-performance liquid chromatography demonstrated that peak immunoreactivity eluted in the same position as the synthetic tetrapeptide. Brain concentrations of the peptide were not reliably different between SHR and WKY. The diurnal rhythm was particularly evident in SHR: the highest concentrations of peptide in both brain and plasma occurred at 2000 hours. These results suggest the presence of another difference between SHR and WKY. (Hypertension 8: 198-202, 1986)

KEY WORDS • hypertension • peptide • naloxone • opiate • radioimmunoassay • high-performance liquid chromatography • corticotropin releasing hormone • Tyr-MIF-1

The reports of alterations in the sensitivity to pain of spontaneously hypertensive rats (SHR) in comparison with genetically matched normotensive Wistar-Kyoto rats (WKY) have been reviewed recently.1 Similar findings have been reported in hypertensive humans.2 The SHR show a decreased sensitivity to pain induced by heat or paw pinch but an increased sensitivity when pain is induced by electric shock.1,3 Therefore, the concentrations of an endogenous antiopiate in SHR might be expected to be different from those in WKY, although the direction of the difference would be difficult to predict.

Tyrosine-MIF-1 (Tyr-Pro-Leu-Gly-NH₂) and MIF-1 (Pro-Leu-Gly-NH₂) have been shown to act like naloxone in some14-17 but not all15-17 studies, and recent results indicate that Tyr-MIF-1 binds to a specific opiate receptor.18 Tyr-MIF-1 has been demonstrated in brain tissue by radioimmunoassay (RIA)17 and high-performance liquid chromatography (HPLC),19 and its concentrations in brain appear to show a diurnal rhythm.17 The presence of saturable, specific, high-affinity bindings sites for Tyr-MIF-1 has also been shown in rat brain.30 Accordingly, the immunoreactive concentrations of Tyr-MIF-1 were measured in plasma and brain of SHR at different times of day and compared with concentrations in the WKY.

Methods and Materials

Male SHR and WKY weighing 180 to 200 g on arrival were obtained from Harlan-Sprague-Dawley (Indianapolis, IN, USA) and allowed free access to food and water. They were housed in a room adjacent to the laboratory for 1 week during a 12 hour light, 12 hour dark cycle (lights on at 0600). All decapitations occurred in the laboratory; a dim red light was used to see the rats removed during darkness.

Truncal blood was obtained in heparinized, chilled tubes immediately after decapitation. Brain tissue (minus pituitary, hypothalamus, and pineal tissue) was removed and frozen on dry ice. For RIA, brain tissue was thawed, weighed, and placed in 5 ml of cold 0.1 M acetic acid. The tissue was then homogenized for 15 seconds with a Brinkmann Polytron (Westbury, NY, USA) at setting 6, decanted into a polypropylene tube, boiled for 1 minute, chilled in ice, and centrifuged at 29,000 g for 20 minutes at 4°C. The supernatant was lyophilized. Lyophilized samples of brain were
reconstituted in borate buffer (pH 8.4) containing 4% aprotinin (Trasylol; Mobay Chemical, New York, NY, USA), 0.1% bovine serum albumin, and 0.002% sodium azide and diluted to an initial concentration of 20 mg/100 μl. Plasma samples for Tyr-MIF-1 were diluted to an initial concentration of 12.5 μl of plasma per 100 μl of buffer. Our own antibodies to Tyr-MIF-1 and oxytocin were used for the RIA at final concentrations of 1:30,000 and 1:15,000. At 32 pg per tube, slightly higher than the midrange of the Tyr-MIF-1 RIA, the interassay coefficient of variation from 10 assays was 5.6% and the intraassay coefficient of variation was 4.1%. Extraction of brain resulted in about 86% recovery of added Tyr-MIF-1. Plasma was assayed unextracted and reflected about 98% of added tetrapeptide.

At each time, 5 SHR and 5 control WKY were used. Two extra WKY were available and were placed in the 1200 and 2000 hours groups. Motor activity was not recorded. No plasma values and only one brain value was discarded as an extreme outlier for statistical analysis. Analysis of variance was followed by Duncan’s multiple range test.

Plasma samples of SHR were analyzed by HPLC to ascertain whether the immunoreactivity being measured by our antibody (number 112) to Tyr-MIF-1 measured material with this structure. This analysis was done with a Beckman system (Model 344; Palo Alto, CA, USA) equipped with Model 100A pumps, Altex Model 210 A sample loop, and Ultrasphere ODS column. The remainder of the system has been described elsewhere. Gradient elution was obtained with aqueous 0.1% trifluoroacetic acid and acetonitrile. The system was equilibrated in 2% acetonitrile and 0.1% trifluoroacetic acid. Two minutes later, the acetonitrile was increased to 35% over 30 minutes, then increased to 100% over 1 minute, maintained at 100% for 8 more minutes, and decreased to 2% for the last 2 minutes. Calibration of the system involved injection of 250 μl of 0.1% trifluoroacetic acid followed by RIA to check for the shadowing artifact.

Results

The results revealed that SHR had higher concentrations of Tyr-MIF-1–like immunoreactivity in their plasma than did the control WKY. Analysis of variance showed a significant main effect of type of rat (SHR vs WKY; \( F_{1,50} = 75.51, p < 0.0001 \)) and time of day \( (F_{5,50} = 5.30, p < 0.001) \). The interaction between type of rat and time of day was not significant. As shown in Figure 1, plasma concentrations of the peptide were higher in SHR than the WKY at 0800 \( (p < 0.05) \), 1200 \( (p < 0.05) \), 1600 \( (p < 0.01) \), 2000 \( (p < 0.0001) \), and 2400 \( (p < 0.01) \), and 0400 \( (p < 0.01) \).

In SHR, plasma concentrations of Tyr-MIF-1–like immunoreactivity were significantly lower at 0800 than at 1600 \( (p < 0.05) \), 2000 \( (p < 0.01) \), and 0400 \( (p < 0.01) \). The difference in plasma concentrations between 0800 and 2400 did not reach statistical significance \( (p = 0.053) \). Immunoreactive plasma concentrations of peptide at 1200 in SHR also were significantly lower than those at 1600 \( (p < 0.01) \), 2000 \( (p < 0.05) \), and 0400 \( (p < 0.05) \). The only times at which plasma concentrations of Tyr-MIF-1–like immunoreactivity in WKY differed significantly were 0800 compared with 1600 \( (p < 0.05) \).

The brain concentrations of Tyr-MIF-1–like immunoreactivity are shown in Figure 2. They were not significantly different between SHR and WKY. There was a significant effect of time of day on the concentrations of peptide in the brain \( (F_{5,49} = 4.16, p < 0.01) \) but no interaction of time by type of rat. In brains of SHR, concentrations of immunoreactive peptide at 2000 were significantly higher than at 0800 \( (p < 0.05) \), 1200 \( (p < 0.01) \), 2400 \( (p < 0.05) \), and 0400 \( (p < 0.05) \). In brains of WKY, concentrations of immunoreactive peptide levels were significantly higher at 1600 than at 0800 \( (p < 0.05) \) but at no other times.

The mean plasma concentration of Tyr-MIF-1–like immunoreactivity for all SHR was 1638.16 ± 73.47 pg/ml, and that for all WKY was 1009.60 ± 41.64 pg/ml (see Figure 1 inset). This 62% difference was

Figure 1. Mean (± SEM) concentrations of Tyr-MIF-1–like immunoreactivity in the plasma of SHR and WKY, of which five per group were decapitated every 4 hours for 24 hours. Inset represents the combined values.
statistically significant \( (p < 0.0001) \). The comparable combined brain concentration for SHR was 0.109 ± 0.018 pg/mg and for WKY was 0.098 ± 0.012 pg/mg (see Figure 2 inset); this 11% difference was not statistically significant.

Since antibody 112 to Tyr-MIF-1 cross-reacts about 5.4% with oxytocin, the C-terminal of which is MIF-1, a maximum of 88.5 pg/ml of the mean of 1638 pg/ml for the SHR might have been due to oxytocin. The 88.5 pg/ml represents only 14% of the mean difference between the peptide concentrations in the plasma of SHR and WKY. Although concentrations of oxytocin-like immunoreactivity in unextracted plasma were at the lower limits of detection in the RIA used in these studies, no plasma samples could have contained more than 20 pg/ml of immunoreactive oxytocin and almost all probably contained less than 12 pg/ml. Vasopressin cross-reacts less than 1% with our Tyr-MIF-1 antibody.

The highest peak of immunoreactive Tyr-MIF-1-like activity after HPLC (Figure 3) of plasma was found to elute at the same position as the Tyr-MIF-1 standard (15.1). The elution positions of oxytocin (21.7) and MIF-1 (6.2) were completely different.

**Discussion**

Our results indicate that the plasma concentrations of Tyr-MIF-1-like immunoreactivity are higher in SHR than in WKY. Results of HPLC indicate that this material represents the Tyr-MIF-1 tetrapeptide (see Figure 3). Peptide concentrations were significantly higher in SHR at each of the six times tested. The concentrations at 0800 and 1200 were significantly lower than at several other times, which demonstrates a diurnal rhythm in plasma that can be seen in Figure 1. This rhythm was particularly evident in the SHR.

The highest concentrations of Tyr-MIF-1-like immunoreactivity in brain tissue occurred at 1600 and 2000, as was found for plasma. The low concentrations of immunoreactive Tyr-MIF-1 in brain tissue at 0800 and 1200 have also been reported in a different strain of rats, but the concentrations at each time were not identical in the two studies. The peptide concentrations in the brains of the control WKY appeared lower than those in the Sprague-Dawley-derived rats obtained from Zivic-Miller used in the previous study. Thus, caution must be exerted in interpreting the differences between SHR and the genetically matched normotensive WKY.

The occurrence of the highest concentrations of Tyr-MIF-1-like immunoreactivity at similar times in both brain and plasma suggests the possibility of increased synthesis, not necessarily from a single location. If the increased concentrations of Tyr-MIF-1-like immunoreactivity in brain represented only increased release from brain tissue with no change in the rate of synthesis, then brain concentrations would be expected to be lower at the same times. Obviously, the concentration of a peptide is the result of several processes that can occur in different combinations and at different sites.
It is not known whether the altered plasma concentrations of immunoreactive Tyr-MIF-1 found in SHR, with altered sensitivities to pain, indicate an involvement of this tetrapeptide in hypertension. Even if this involvement did exist, it would not be clear whether it was related to the antinociceptive activity of Tyr-MIF-1. The absence of significant changes in brain concentrations emphasizes this point, although changes in a small part of the brain could have been masked by the assay of whole brain. Opiate peptides are known to exert nonanalgesic effects, as we showed in 1976, and can affect blood pressure in both SHR and normotensive rats.

Many of the actions of MIF-1 and Tyr-MIF-1, however, do not involve antinociceptive effects. Since MIF-1 and Tyr-MIF-1 are active in animal models in which dopa and tricyclic antidepressants are also effective, the differences in peptide concentrations between SHR and WKY could reflect basic differences in catecholaminergic mechanisms. It is not known whether such possible interactions of Tyr-MIF-1 would also involve the opiate peptides with which catecholamines can be anatomically associated in the brain and periphery.

There are indications that catecholamines can be released by corticotropin-releasing hormone. This hormone stimulates the release of the opiate beta-endorphin, corticotropin, and melanocyte-stimulating hormone from the pituitary. In fact, MIF-1 originally was named for its action in inhibiting the release of melanocyte-stimulating hormone, an action evident only under limited experimental conditions. A recent article includes unpublished observations of a decreased corticotropin response to corticotropin-releasing factor in SHR as compared with that in WKY, and plasma beta-endorphin levels have been reported to be decreased in SHR. Preliminary experiments in our laboratory indicate that MIF-1 and Tyr-MIF-1 may inhibit corticotropin-releasing factor–induced corticotropin release.

Although the relationship of corticotropin-releasing factor, opiates, and antidiabetics to hypertension remains unclear, our findings suggest that plasma Tyr-MIF-1 concentrations are higher in SHR than in WKY.

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