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BRIEF COMMUNICATION

Tyr-MIF-1 Augments Benzodiazepine Receptor Binding *In Vivo*

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MILLER, L. G., A. J. KASTIN AND D. J. GREENBLATT. *Tyr-MIF-1 augments benzodiazepine receptor binding* in vivo. PHARMACOL BIOCHEM BEHAV **28**(4) 521–524, 1987.—Behavioral and limited neurochemical evidence indicates possible links between the endogenous opiate and γ-aminobutyric acid (GABA)-benzodiazepine receptor systems. A previous study using *in vitro* techniques indicated that MIF-1 (Pro-Leu-Gly-NH_2) and Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2), peptides with anti-opiate activity, enhanced GABA-stimulated benzodiazepine binding. To assess the activity of these peptides under *in vivo* conditions, we evaluated their effects on benzodiazepine receptor binding as determined by specific uptake of the benzodiazepine antagonist [^{3}H]-Ro15-1788. Tyr-MIF-1, at a dose of 1 mg/kg IP, significantly augmented benzodiazepine binding in cortex and hippocampus but not in cerebellum, hypothalamus, or pons-medulla. Increases in binding were due in large part to increased apparent affinity at the receptor. At none of the doses of MIF-1 (0.1, 1 and 10 mg/kg) or at the highest (10 mg/kg) and lowest (0.1 mg/kg) doses of Tyr-MIF-1 was there any significant alteration in benzodiazepine binding in any region evaluated. These results indicate that peptide-benzodiazepine receptor interactions may also occur *in vivo*.

MIF-1 Tyr-MIF-1 Benzodiazepine GABA Receptor

ENDOGENOUS opiate and GABA-benzodiazepine systems have been described in detail during the past decade [8,21]. Although the two systems have distinct receptors and pharmacologic specificity, behavioral evidence suggests a link between them. Several studies have reported that opiate antagonists may also have antagonistic effects in the GABA-benzodiazepine system [1–3], and the converse has been reported for benzodiazepine antagonists [6]. Some evidence from neurochemical studies also supports an association between these systems [9, 19, 20].

In parallel with the endogenous opiate systems, there is evidence for an anti-opiate system that appears to function in balance [5]. MIF-1 (Pro-Leu-Gly-NH_2) and Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) are small brain peptides with anti-opiate properties, such as antagonism of stress-induced and opiate-induced analgesia and ingestive behavior [6, 10, 11, 17]. Because of possible effects of opiate antagonists on benzodiazepine-induced behavior, we examined the effects of these peptides on benzodiazepine binding in mouse brain synaptosomal preparations [12]. Both peptides enhanced GABA-stimulated benzodiazepine binding *in vitro* in several brain regions, probably by increasing apparent affinity at the benzodiazepine receptor.

As we and several other groups have shown, benzodiazepine binding characteristics *in vitro* may not reflect binding *in vivo* [4, 7, 13, 15], and some evidence indicates that *in vivo* binding correlates more closely with physiologic function [13]. We assessed the effects of MIF-1 and Tyr-MIF-1 on benzodiazepine receptor binding *in vivo*, using recently described methods based on specific uptake of [^{3}H]-Ro15-1788 [7,13].

METHOD

Male CD-1 mice, 6–8 weeks of age, were obtained from

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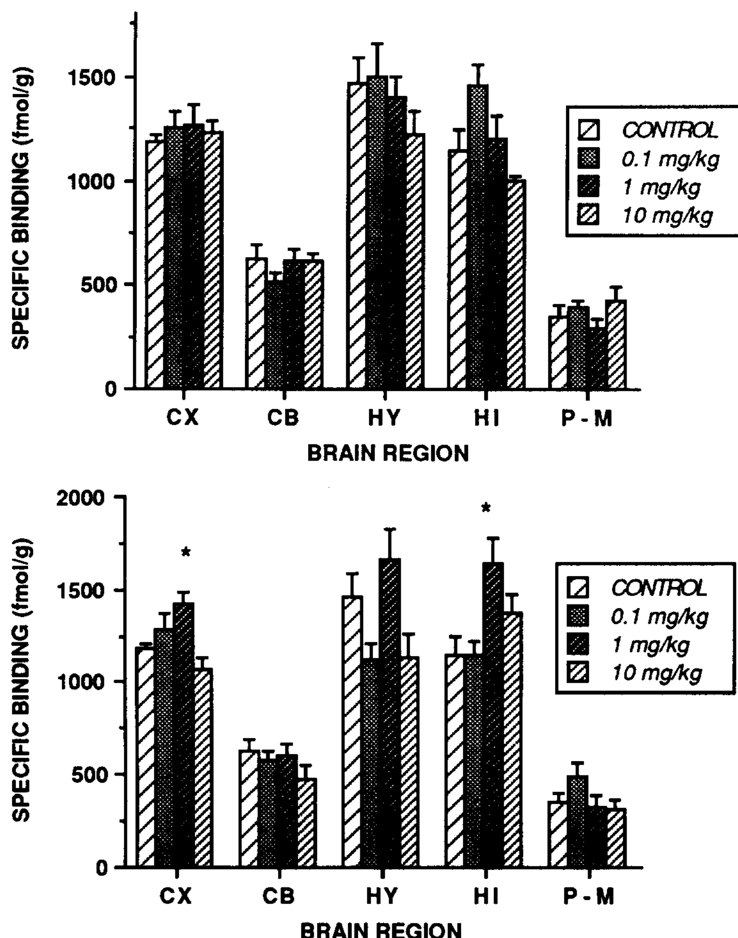

FIG. 1. Effects of MIF-1 and Tyr-MIF-1 on specific uptake of [^{3}H]-Ro15-1788 *in vivo*. Binding was determined 25 min after injection of peptide. CX=cortex, CB=cerebellum, HY=hypothalamus, HI=hippocampus, P-M=pons-medulla. Top: MIF-1. Results are mean±SEM, n=8 at 0.1 and 1 mg/kg, n=6 at 10 mg/kg, n=7 for controls. Bottom: Tyr-MIF-1. Results are mean±SEM, n=8 at 0.1 and 1 mg/kg, n=5 at 10 mg/kg, n=7 for controls. *=$p<0.05$ vs. controls.

Charles River Laboratories (Wilmington, MA), housed under a 12 hr light-dark cycle, and allowed free access to laboratory chow and water. [^{3}H]-Ro15-1788 (specific activity 81 Ci/mmol) and Protosol were obtained from New England Nuclear (Boston, MA). Clonazepam and desmethylflunitrazepam were generously provided by Roche Laboratories (Nutley, NJ).

Peptide Administration

MIF-1 and Tyr-MIF-1 were dissolved in saline (0.9% NaCl) and administered IP (0.1–10 mg/kg). Control mice received saline alone. In accordance with behavioral studies indicating peak effect at 20–30 min [5], receptor binding was determined 25 min after administration of peptide or saline.

Benzodiazepine Receptor Binding In Vivo

Receptor binding was determined as previously described [13]. Briefly, 5 min after administration of peptide or vehicle, mice were injected with 3 μCi [^{3}H]-Ro15-1788 in the tail vein. Twenty min later, mice were decapitated and brains rapidly removed. After dissection on ice, brain regions were weighed and placed in scintillation vials containing 3 ml Protosol. Vials were incubated at 4°C for 24 hr. Scintillation fluid, 10 ml, was added to each vial, and vials were allowed to stand for 24 hr before being counted by conventional scintillation spectrometry. To determine nonspecific binding, an additional group of mice received a saturating dose of clonazepam (5 mg/kg IP) concurrent with injection of the peptide. Tissue was processed as above, and nonspecific binding was subtracted from total binding in each region to yield specific binding.

Benzodiazepine Receptor Affinity

Apparent affinity at the receptor was determined as previously described [13]. Briefly, mice received varying doses of clonazepam (0.02–2 mg/kg) concurrent with injection of the peptide and tissue was processed as above, except that cortices were divided. One segment was used for receptor binding analysis as above, and the other segment was placed in 1 ml 0.025 M borate buffer (pH 8.3), homogenized with a Polytron (Brinkmann, Lucerne; setting 7, 4 pulses), and

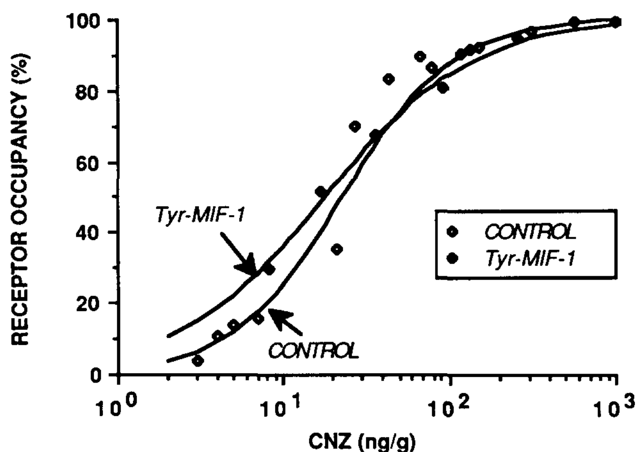

FIG. 2. Clonazepam (CNZ) concentration versus receptor occupancy in cortex of mice treated with Tyr-MIF-1 (1 mg/kg). Varying doses of CNZ (0.2–2 mg/kg) were injected IP concurrently with Tyr-MIF-1. Binding and CNZ concentrations were determined after 25 min. Each point represents the mean of 2 mice for Tyr-MIF-1 and 3 mice for controls. The IC_{50} values (mean±SD) are: Tyr-MIF-1, 17.9±1.4 ng/g; control, 22.8±1.5 ng/g, $p<0.05$.

analyzed for clonazepam by gas-liquid chromatography [14]. Receptor occupancy was calculated as previously described [13], that is, specific binding per tissue weight of clonazepam-treated mice divided by specific binding per tissue weight of vehicle-treated mice. Data were fit to the modified Hill equation: $y = x^A/[(IC_{50}^A) + x^A]$, where x= clonazepam concentration in ng/g and y=receptor occupancy in percent.

Statistical Analyses

Data were analyzed by analysis of variance (ANOVA) followed by Scheffe's test for multiple comparisons.

RESULTS

MIF-1 (0.1–10 mg/kg) had little effect on benzodiazepine receptor binding in any of the five brain regions evaluated (Fig. 1, top). By contrast, Tyr-MIF-1 augmented benzodiazepine receptor binding in cortex, $F(3,26)=3.1$, $p<0.05$, and hippocampus, $F(3,23)=6.7$, $p<0.01$, but not in cerebellum, hypothalamus, or pons-medulla (Fig. 1, bottom). Comparison tests showed that this effect was due to the 1 mg/kg dose, which resulted in a significantly greater increase than did the control in both cortex ($p<0.05$) and hippocampus ($p<0.01$). Neither peptide altered nonspecific binding at any of the doses evaluated.

The observed increases in benzodiazepine binding could be due to increases in receptor number or in affinity at the receptor. To distinguish these possibilities, apparent affinity for clonazepam was determined in cortices of mice treated with Tyr-MIF-1 (1 mg/kg) and in controls (Fig. 2). The IC_{50} value for treated mice was 17.9±1.4 ng/g compared to 22.8±1.5 ng/g for controls (mean±SD, $p<0.05$). Thus, the increase in receptor binding was due in large part to an increase in apparent affinity at the receptor.

DISCUSSION

Previous results obtained with *in vitro* synaptosomal preparations indicated that both MIF-1 and Tyr-MIF-1 augmented benzodiazepine binding in the presence of exogenous GABA, but had little effect in well-washed membrane preparations without added GABA [12]. These results suggested that the peptides did not affect the benzodiazepine binding site directly, but rather altered the coupling between the GABA and benzodiazepine binding sites [8]. Results from the present study confirm the findings for Tyr-MIF-1 *in vitro* and extend them to benzodiazepine binding in the presence of endogenous GABA.

Increases in binding in cortex were in large part due to increased apparent affinity at the receptor, in agreement with the *in vitro* results [12]. Changes in binding were not observed in cerebellum or pons-medulla, again in accordance with the *in vitro* findings. In addition, increased binding was observed in the hippocampus, a region not examined in the *in vitro* studies. The effects of Tyr-MIF-1 across the dose range used appeared to have an inverted U-shape, with little change in binding at the highest dose (10 mg/kg). This pattern has been described for the effects of Tyr-MIF-1 in other systems [5].

In contrast to the results with Tyr-MIF-1, no change in binding was observed with MIF-1 *in vivo*, despite enhancement of GABA-stimulated binding *in vitro*. This is unlikely to be due to changes in delivery to brain, since behavioral studies indicate efficacy of both peptides injected in the periphery at the doses used in this study [11]. In the *in vitro* studies, MIF-1 tended to be less potent than Tyr-MIF-1 across a broad range of doses, and neither peptide had significant effects at higher concentrations of GABA. It is possible that the effects of MIF-1 *in vivo* are masked in the presence of endogenous GABA concentrations. Alternatively, both compounds may act through opiate receptors that could be differentially available or activated in *in vivo* as compared to *in vitro* preparations [21].

Other neurochemical evidence supports a relation between the opiate and GABA-benzodiazepine systems. Acute benzodiazepine administration has been reported to modulate release of endogenous opiates in several brain regions [9]. In addition, altered benzodiazepine receptor binding was observed after chronic treatment with morphine [19,20]. The present results, together with previous results from *in vitro* studies, indicate that the brain peptide Tyr-MIF-1 augments benzodiazepine receptor binding. Several mechanisms could be postulated for this action, including direct action at the benzodiazepine/GABA complex, interaction with opiate receptors [21,22] or action at the Tyr-MIF-1 binding site [21]. Regardless, this effect may contribute to the behavioral linkages observed between benzodiazepine, opiate, and other peptide systems.

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