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BRIEF COMMUNICATION

Interactions of Tyr-MIF-1 at Opiate Receptor Sites

JAMES E. ZADINA[1] AND ABBA J. KASTIN

Veterans Administration Medical Center
and Tulane University School of Medicine, New Orleans, LA 70146

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ZADINA, J. E. AND A. J. KASTIN. *Interactions of Tyr-MIF-1 at opiate receptor sites.* PHARMACOL BIOCHEM BEHAV **25**(6) 1303–1305, 1986.—Binding of Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) to mu and delta opiate receptors was compared with other putative opiate antagonist peptides by displacement of iodinated ligands selective for mu (DAGO, FK33824, and morphiceptin) and delta (DPDPE) receptors. Tyr-MIF-1 and ACTH (1–24 and 1–39) inhibited binding of ^{125}I-DAGO with IC_{50}'s of about 1 μM. FMRF-NH_2 was about an order of magnitude weaker while CCK-8 and MIF-1 failed to inhibit 50% of binding at concentrations up to 100 μM. Morphiceptin, Tyr-MIF-1, and ACTH were less potent but more efficacious than DAGO, FK33824, morphine, or naloxone in inhibiting the binding of ^{125}I-morphiceptin. Tyr-MIF-1 appeared to have a more selective action at opiate receptors than ACTH; in contrast to their effects at ^{125}I-DAGO-labeled sites, morphiceptin and Tyr-MIF-1 inhibited less than 50% of ^{125}I-DPDPE binding at concentrations up to 10 and 50 μM, while ACTH 1–39 and 1–24 inhibited more than 80% of the binding at 2.5 and 5 μM, respectively. The results indicate that at relatively high concentrations Tyr-MIF-1, like ACTH, can affect binding to the opiate receptor, but unlike ACTH, binding of Tyr-MIF-1 appears relatively selective for the mu site.

ACTH CCK DAGO DPDPE FK33824 FMRF-NH_2 MIF-1 Morphiceptin
Opiate receptors Mu Delta Tyr-MIF-1

SEVERAL endogenously occurring peptides have been postulated to act as opiate antagonists (for review, see [6]). One of these peptides, Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2), has been shown to block opiate actions in animal models of thermal and chemical pain [9,10], stress-induced analgesia [7], and to block long-term nociceptive changes observed after neonatal administration of opiate peptides [20]. Two of the many possible mechanisms by which Tyr-MIF-1 may cause opiate antagonism include binding to a receptor specific for substances similar to Tyr-MIF-1 and binding to an opiate receptor. High affinity binding sites for Tyr-MIF-1 have been demonstrated in brain [17,19]. Displacement studies using ^{125}I-Tyr-MIF-1 indicate structural specificity of these sites for substances similar to Tyr-MIF-1 or the beta-casomorphin family of milk-derived opiate-like peptides, including morphiceptin (Tyr-Pro-Phe-Pro-NH_2) [19]. Since the binding of radioactive morphiceptin to its sites in brain can be displaced by Tyr-MIF-1, it appears that the two substances share a binding site or allosterically inhibit binding of each other to their respective sites [19]. Morphiceptin also binds selectively to mu opiate receptors as indicated by a high ratio of potency in displacing mu relative to delta opiate receptor ligands [2, 3, 8].

Besides binding at its own site, Tyr-MIF-1 could bind to opiate receptor sites. We tested this possibility using iodinated DAGO (Tyr-D-Ala-Gly-(Me)Phe-Gly-ol), FK33824 (Tyr-D-Ala-Gly-(Me)Phe-Met-ol), and morphiceptin, all of which have high selectivity for the mu opiate receptor site, or the enkephalin analog DPDPE (D-Pen^2, D-Pen^5-enkephalin), which has a high selectivity for delta over mu opiate receptors [12].

METHOD

DAGO, morphiceptin, DPDPE, and FK33824 were iodinated with chloramine T and were purified by HPLC to isolate the monoiodinated fraction. The specific activity of the ligands was 1900–2100 Ci/mMole.

Male Sprague-Dawley-derived rats were obtained from Zivic-Miller (Allison Park, PA) and whole brains minus cerebellum were removed and homogenized in 0.32 M sucrose. After centrifugation at 1000 g for 10 min, the supernatant was centrifuged at 30,000 g for 15 min. The resulting pellet was reconstituted in 10 mM Tris and incubated for 30 min at room temperature to allow dissociation of endogenous ligands. The homogenates were centrifuged, reconstituted in

[1]Requests for reprints should be addressed to James E. Zadina, VA Medical Center, 1601 Perdido Street, New Orleans, LA 70146.

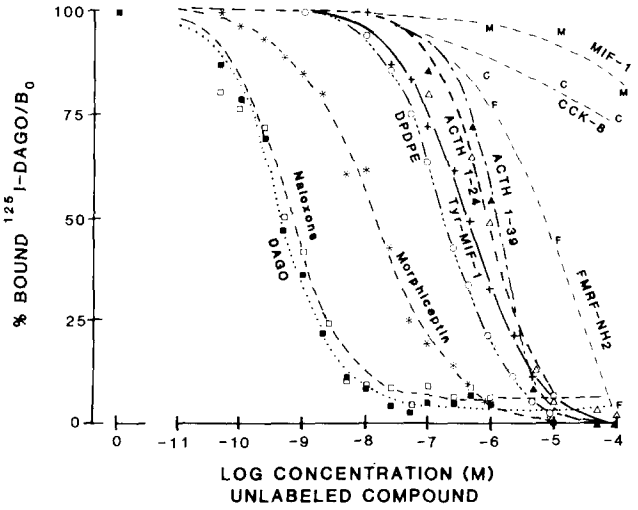

FIG. 1. Displacement of ^{125}I-DAGO (0.2 nM) by DAGO, naloxone, morphiceptin, DPDPE, Tyr-MIF-1, ACTH 1–39, ACTH 1–24, FMRF-NH_2, CCK-8, and MIF-1. Curves were generated by the ALLFIT program.

the same buffer, and allowed to incubate again for 30 min at room temperature before a final centrifugation and reconstitution in 50 mM Tris. The membranes (500 μg protein/ml) were incubated at room temperature for 1 hour with 0.20–0.35 nM iodinated peptide in the presence or absence of unlabeled peptides obtained from Bachem or Peninsula laboratories. Nonspecific binding was defined as that observed in the presence of a 10 μM concentration of the unlabeled peptide. A cell harvester modified for receptor binding and Whatman GF/B filters were used for separation of bound and free ligand as previously described [18]. Data were analyzed with the ALLFIT program [4] for determination of IC_{50} values.

RESULTS

As expected, both DAGO and naloxone showed potent inhibition of ^{125}I-DAGO binding to rat brain (Fig. 1). Both of these compounds had IC_{50}'s below 1 nM (0.47 and 0.58 nM). In separate experiments, morphine sulfate and FK-33824 also displaced ^{125}I-DAGO with IC_{50}'s below 1 nM. Morphiceptin, which has high selectivity for mu over delta opiate receptors, was less potent than DAGO or naloxone and displaced ^{125}I-DAGO with an IC_{50} of 13.7 nM, in good agreement with previously reported values for this compound [3]. The selective delta agonist DPDPE inhibited 50% of ^{125}I-DAGO binding at 192 nM, resulting in a ratio of 0.0024 for the concentrations of DAGO/DPDPE at their IC_{50}'s, in good agreement with a previous report of competition of the related compound DPLPE, for ^{125}I-FK33824 binding [13].

Tyr-MIF-1 and both ACTH 1–39 and ACTH 1–24 produced half-maximal inhibition of ^{125}I-DAGO binding at concentrations near micromolar levels (Fig. 1). IC_{50} values were 0.44, 0.92 and 1.18 micromolar for Tyr-MIF-1, ACTH 1–24, and ACTH 1–39.

Three other peptides with putative antiopiate properties were less effective or ineffective in displacing ^{125}I-DAGO. While concentrations near 10 μM FMRF-NH_2 were required for 50% inhibition, CCK-8 and MIF-1 were ineffective at achieving this level of inhibition even at ten times that concentration (Fig. 1). These results confirm our earlier report of no inhibition of ^{3}H-naloxone or ^{3}H-D-Ala^2-Leu-enkephalin binding by MIF-1 [11].

Inhibition of ^{125}I-FK33824 by DAGO, naloxone, morphine, morphiceptin, and Tyr-MIF-1 was similar to that observed with ^{125}I-DAGO. For ^{125}I-morphiceptin binding, naloxone, morphine sulfate, FK33824, and DAGO all inhibited 40–50% of the specific binding at concentrations below 10 nM, but tended to show little additional inhibition at higher concentrations. In contrast, morphiceptin, Tyr-MIF-1, ACTH 1–39, and ACTH 1–24 inhibited 70% of the binding at concentrations between 0.1 and 1.0 μM.

^{125}I-DPDPE displacement by naloxone, DAGO and unlabeled DPDPE was consistent with the known pattern of selectivity and affinity of this enkephalin analog for opiate receptors; IC_{50}'s for DPDPE and naloxone were about 10 nM while half-maximal inhibition by DAGO occurred above 100 nM. Tyr-MIF-1 and morphiceptin on the one hand, and ACTH 1–24 and 1–39 on the other hand, however, showed distinctly different patterns of inhibiting ^{125}I-DPDPE binding; while all 4 compounds inhibited 30% of the binding between 0.1 and 1.0 μM, very little additional inhibition was observed with higher concentrations of Tyr-MIF-1 or morphiceptin. A 50% inhibition of binding was not observed with the highest concentrations tested of 10 μM for morphiceptin and 50 μM for Tyr-MIF-1. Increasing concentrations of ACTH 1–24 and 1–39, however, continued to decrease ^{125}I-DPDPE binding, inhibiting more than 80% of the binding at 2.5 and 5.0 μM, respectively.

DISCUSSION

The effects of opiates can be blocked under several experimental conditions by Tyr-MIF-1 [6, 7, 9, 10] and by ACTH [1, 5, 15]. ACTH in relatively high concentrations has previously been reported to inhibit binding of opiate ligands to their receptor sites [14, 15]. The present study extends these previous reports by the use of ligands selective for mu and delta opiate receptor subtypes. In addition, Tyr-MIF-1 in similar concentrations (about 1 μM) is shown to inhibit binding to opiate receptors labeled with radioactive DAGO and FK33824 and confirms our previous report [19] that specific morphiceptin binding in brain is displaced by Tyr-MIF-1. Tyr-MIF-1 is similar in potency to ACTH in binding to opiate receptor sites, but it appears to be more selective since ACTH inhibits binding at sites labeled with both mu and delta ligands. Although the effects of Tyr-MIF-1 may be mediated at its own binding site, the present results indicate the possibility that Tyr-MIF-1 may act at opiate receptor sites.

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REFERENCES

1. Bertolini, A., R. Poggioli and W. Ferrari. ACTH-induced hyperalgesia in rats. *Experientia* **35:** 1216–1217, 1979.
2. Chang, K. J., A. Killian, E. Hazum and P. Cuatrecasas. Morphiceptin (NH_4-Tyr-Pro-Phe-Pro-$CONH_2$): A potent and specific agonist for morphine (μ) receptors. *Science* **212:** 75–77, 1981.
3. Chang, K.-J., E. Hazum and P. Cuatrecasas. Novel opiate binding sites selective for benzomorphan drugs. *Proc Natl Acad Sci USA* **78:** 4141–4145, 1981.
4. DeLean, A., P. J. Munson and D. Rodbard. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am J Physiol* **235:** E97–E102, 1978.
5. Galina, Z. H. and Z. Amit. Interactions between ACTH, morphine, and naloxone and their effects on locomotor behavior. *Prog Neuropsychopharmacol Biol Psychiatry* **9:** 691–695, 1985.
6. Galina, Z. H. and A. J. Kastin. Minireview: Existence of antiopiate systems as illustrated by MIF-1/Tyr-MIF-1. *Life Sci* **39:** 2153–2159, 1986.
7. Galina, Z. H. and A. J. Kastin. Tyr-MIF-1 attenuates antinociceptive responses induced by three models of stress-induced analgesia. *Br J Pharmacol*, in press.
8. James, I. F. and A. Goldstein. Site-directed alkylkation of multiple opioid receptors. I: Binding selectivity. *Mol Pharmacol* **25:** 337–342, 1984.
9. Kastin, A. J., E. Stephens, R. H. Ehrensing and A. J. Fischman. Tyr-MIF-1 acts as an opiate antagonist in the tail-flick test. *Pharmacol Biochem Behav* **21:** 937–941, 1984.
10. Kastin, A. J., E. Stephens, J. E. Zadina, D. H. Coy and A. J. Fischman. Tyr-MIF-1, identified in brain tissue, and its analogs are active in two models of antinociception. *Pharmacol Biochem Behav* **23:** 1045–1049, 1985.
11. Luciano, M. G., J. E. Zadina, A. J. Kastin and D. H. Coy. Mu and delta opiate receptors in rat brain are affected by GTP but not by MIF-1. *Brain Res Bull* **7:** 677–682, 1981.
12. Mosberg, H. I., R. Hurst, V. J. Hruby, K. Gee, H. I. Yamamura, J. J. Galligan and T. F. Burks. Bis-penicillamine enkephalins possess highly improved specificity toward δ opioid receptors. *Proc Natl Acad Sci USA* **80:** 5871–5874, 1983.
13. Moyse, E., F. Pasquini, R. Quirion and A. Beaudet. ^{125}I-FK33-824: A selective probe for radioautographic labeling of μ opioid receptors in the brain. *Peptides* **7:** 351–355, 1986.
14. Stengaard-Pedersen, K. and L.-I. Larsson. Localization and opiate receptor binding of enkephalin, CCK and ACTH/β-endorphin in the rat central nervous system. *Peptides* **2:** Suppl 1, 3–19, 1981.
15. Terenius, L. Effect of peptides and amino acids on dihydromorphine binding to the opiate receptor. *J Pharm Pharmacol* **27:** 450–452, 1975.
16. Terenius, L., W. H. Gispen and D. deWied. ACTH-like peptides and opiate receptors in the rat brain: structure-activity studies. *Eur J Pharmacol* **33:** 395–399, 1975.
17. Zadina, J. E., A. J. Kastin, E. F. Krieg, Jr. and D. H. Coy. Characterization of binding sites for N-Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) in rat brain. *Pharmacol Biochem Behav* **17:** 1193–1198, 1982.
18. Zadina, J. E. and A. J. Kastin. A rapid filtration method for receptor binding: Characterization with mu and delta opiate receptors. *Pharmacol Biochem Behav* **21:** 947–951, 1984.
19. Zadina, J. E. and A. J. Kastin. Interactions between the antiopiate Tyr-MIF-1 and the mu opiate morphiceptin at their respective binding sites in brain. *Peptides* **6:** 965–970, 1985.
20. Zadina, J. E., A. J. Kastin, P. K. Manasco, M. F. Pignatiello and K. L. Nastiuk. Long-term hyperalgesia induced by neonatal beta-endorphin and morphiceptin is blocked by neonatal Tyr-MIF-1. *Brain Res*, in press.