European Journal of Pharmacology, 91 (1983) 327–328
Elsevier

Rapid communication

[^{3}H]DYNORPHIN A BINDS SELECTIVELY TO GUINEA PIG BRAIN

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Received 14 June 1983, accepted 21 June 1983

Dynorphin A effectively displaces prototypical ligands for the κ type of opiate receptors from rat and guinea pig brain membranes. Similar findings from studies utilizing peripheral organs suggest that dynorphin A is an endogenous ligand for the κ-receptor (Chavkin et al., 1982; Corbett et al., 1982). However, dynorphin A shows substantial potency in displacing prototypical agonists for the μ- and δ-receptors ([^{3}H]morphine and [^{3}H][D-Ala^2,D-Leu^5]enkephalin (DADLE)). Because the usual radiolabeled ligands for opiate receptor types lack complete selectivity, it is unclear whether dynorphin competes at the μ and δ sites or if these radioligands bind to the κ-receptor. In addition, a number of nonopiate effects of dynorphin have been reported suggesting that dynorphin might bind to receptors that are unaffected by opiates (Walker et al., 1982; Przewlocki et al., 1983). These nonopiate effects are equally well produced with des-tyrosine-dynorphin (dT-Dyn), a finding analogous to the nonopiate effects reported for des-Tyr-γ-endorphin (De Wied et al., 1978). Until now a full characterization of dynorphin binding including a possible nonopiate receptor has been impossible because radiolabeled dynorphin has not been available. We now report that [^{3}H]dynorphin A binds specifically to guinea pig brain membranes and appears to selectively label κ sites.

[^{3}H]Dynorphin A-(1-17) (synthesized by RH) exhibited an extremely high proportion of nonspecific binding in preliminary studies on crude membrane preparations of rat brain (RH, in preparation). Consequently, studies were undertaken with crude membrane preparations of guinea pig brain minus cerebellum. The brains were homogenized with a Brinkman polytron at a concentration of 50 mg tissue/ml of 0.05 M Tris buffer, pH 7.4 (25°C). The preparation was preincubated at 37°C for 40 min. It was then centrifuged at 30000 × *g* and resuspended in 0.05 M Tris buffer with 0.2% bovine serum albumen (BSA) at a concentration of 37.5 mg/ml. [^{3}H]Dynorphin A was suspended in 0.05 M Tris buffer. Dynorphin A and UM 1071 standards were added in 10 μl MeOH-0.1 N HCl (1 : 1 mixture). Morphine standards were added in 10 μl water and DADLE standards were added in 10 μl Tris buffer. The total assay volume was 0.5 ml. After 90 min incubation at 0°C (equilibrium) the bound [^{3}H]dynorphin A was separated from free by filtration over Whatman GF/B glass fiber filters presoaked in Tris buffer with 0.4% BSA, and 0.1% polylysine. Each tube was rinsed with 9 ml of 0.05 M Tris buffer containing 0.1% BSA, 0.01% Triton X-100 and 100 mM choline chloride. Approximately 50% of the binding was displaceable by levorphanol but not dextrorphan. dT-Dyn failed to displace any of the binding; in fact, it increased both specific and nonspecific binding proportionately by approximately 10%. Competition curves of dynorphin A, morphine, DADLE and UM 1071 (the active stereoisomer of MR 2034, a presumed κ ligand) against 0.5 nM [^{3}H]dynorphin A are shown in fig. 1.

[^{3}H]Dynorphin A binding shows classic opiate displacement patterns. A prototypical μ-receptor agonist (morphine) and a prototypical δ-receptor agonist (DADLE) were approximately 200 times less potent than the two presumed κ agonists (dynorphin A and UM 1071) in the displacement of [^{3}H]dynorphin A binding. Thus, in guinea pig brain, dynorphin A appears to be the most selective κ ligand reported to date. It is unclear if the

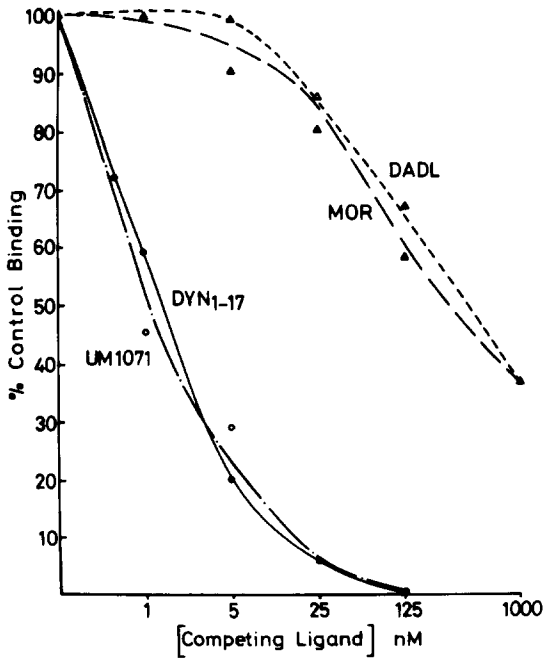

Fig. 1. Relative potencies of μ (morphine = MOR), δ ([D-Ala^2,D-Leu^5]Enkephalin = DADL) and κ (UM1071) ligands in displacing [^{3}H]dynorphin from guinea pig brain membranes. Assays were conducted in triplicate at 4°C with 90 min incubations and separated over Whatman GF/B filters. The IC_{50}s for the μ and δ ligands were 200 times greater than the IC50s for the presumed κ ligands.

50% of [^{3}H]dynorphin A binding that is not displaceable by opiates includes a nonopiate binding site for dynorphin A. However, the failure of dT-Dyn to displace any of the [^{3}H]dynorphin A binding suggests that another mechanism may account for its effects in vivo.

Acknowledgements

This work was supported by grants NIDA Center DA00154, NIDA 02265 and the Theophile Raphael Fund.

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