

[³H] DYNORPHIN BINDING TO GUINEA PIG AND RAT BRAIN

E. Young, J.M. Walker, R. Houghten, H. Akil

Mental Health Research Institute
University of Michigan
Ann Arbor, Michigan 48109

(Received in final form June 26, 1983)

Summary

[³H] Dynorphin can be shown to bind to the brains of both rat and guinea pigs with approximately 50% specific binding. Characterization of the binding in terms of multiple opiate receptor types supports the kappa selectivity of dynorphin in guinea pig. However, in rat brain, a substantial proportion of the [³H] dynorphin binding is displaced by morphine, suggesting a mu as well as kappa component. Consequently, in rat, dynorphin may show effects at both mu and kappa receptors in vivo.

Dynorphin A 1-17 has been reported to be an endogenous kappa ligand (1,2). However, using in vitro brain binding paradigms, dynorphin shows substantial potency in competing for [³H] morphine or [³H] D-ala²-leu⁵ enkephalin (DADL) binding sites. Studies in which dynorphin A 1-17 is competed against [³H] kappa ligands usually require blockade or destruction of the mu and delta sites, and subsequent competition against the remaining kappa sites, since completely selective kappa ligands are not available. While this has proven a very useful approach, it may alter a subpopulation of kappa sites which becomes unavailable for study. Consequently there is a need for more direct studies in which dynorphin is used as the radiolabelled ligand and mu, delta and kappa ligands compete with dynorphin for dynorphin binding sites.

The characterization of dynorphin as a kappa ligand is further confounded by the difficulty in demonstrating the existence of the kappa receptor. Initial studies in rat brain failed to demonstrate a kappa receptor. In contrast, studies with guinea pig brain supported the existence of the kappa receptor. More recent work in rat brain by Pfeiffer et al (3) and Chang et al (4) support the existence of a third site called R₃ or the benzomorphan site. It is unclear if these two sites correspond to the kappa site. We reasoned that some of the discrepancies may be due to species differences between rat and guinea pig brain. Our own data using [³H] MR2034 suggest twice as many kappa sites in guinea pig brain than in rat brain. This difference in the availability of kappa sites may influence the preference of dynorphin for the kappa opiate receptor in rat versus guinea pig. To answer these questions we have undertaken the characterization of [³H] dynorphin binding in both rat and guinea pig brain.

METHODS

[³H] dynorphin of high specific activity (50 Ci/mole) was synthesized by one of us (RH). Binding studies used homogenates of whole brain minus

cerebellum. Brains from both guinea pig and rat were homogenized with a Brinkman Polytron in 0.05 M Tris buffer (pH 7.4 at 25°C) at a concentration of 50 mg tissue/ml buffer. Brain homogenates were incubated at 37°C for 40 minutes, then centrifuged at 30,000 x g. The membrane pellet was resuspended in 0.05 M Tris buffer with 0.2% bovine serum albumen (BSA) at a concentration of 37.5 mg/ml. [³H] dynorphin was suspended in 0.05 M Tris buffer; the concentration in the assay was 0.5 nM. Nonspecific binding was defined by 1 μM levorphanol or 1 μM UM1071 (The active stereoisomer of MR2034, a kappa ligand). Unlabelled dynorphin and UM1071 were added in a small amount (10 microliters) of MeOH-HCl (1:1 mixture Methanol:0.1N HCl). Previous studies have shown that this volume of MeOH:HCl in the final volume of 0.5 ml does not affect the binding of mu, delta or kappa ligands to rat or guinea pig brain. Morphine stocks were dissolved in water and DADL in Tris buffer. Preliminary studies indicated that [³H] dynorphin binding reached equilibrium at 0°C in 60-90 minutes in both rat and guinea pig brain. After a ninety minute incubation period at 0°C, the bound [³H] dynorphin was separated from the free by rapid filtration under vacuum over Whatman GF/E glass fiber filters. The filters were presoaked in 0.05 M Tris buffer with 0.4% BSA and 0.1% polylysine. Each tube was rinsed with 9 ml of ice cold 0.05 M Tris buffer with 0.1% BSA, 0.01% Triton X-100, and 100 mM choline chloride. Using this procedure, the binding of [³H] dynorphin to filters is reduced to less than 10% of total counts added.

All concentrations were run in triplicate. Both guinea pig and rat brain studies were run simultaneously using the same [³H] dynorphin and the same competing ligand stocks under the same incubation conditions to assure differences were not due to changes in labelled or unlabelled ligands over time. Morphine, DADL, dynorphin A 1-17 and UM1071 were competed against [³H] dynorphin binding to characterize the binding in terms of mu, delta, and kappa receptor preference.

RESULTS

[³H] dynorphin binds with approximately 50% specific binding to both species. Both dynorphin A and UM1071 showed substantial ability to displace [³H] dynorphin in rat and guinea pig brain (Table 1).

TABLE I
IC₅₀ of Various Ligands Against 0.5 nM [³H] Dynorphin

	Rat	Guinea Pig
Dynorphin A (1-17)	2.4 nM	1.2 nM
UM1071	2.0 nM	1.1 nM
Morphine	32 nM	680 nM
DADL	300 nM	> 1000 nM

However, there are substantial species differences in the ability of morphine and DADL to compete for [³H] dynorphin binding sites (Figure 1 and 2). In the case of rat brain, the IC₅₀ of morphine against [³H] dynorphin is 32 nM, while under the same conditions, the IC₅₀ of morphine in guinea pig brain is 680 nM. DADL enkephalin also shows a shift in IC₅₀ between species, but this is not as dramatic as the morphine shift. However, the IC₅₀ of DADL is quite high in both species suggesting that dynorphin A shows little preference for the delta receptor. In contrast, in rat brain, dynorphin A shows more morphine displaceable binding and less kappa selectivity. The simplest explanation for this species difference is the number of kappa sites available for [³H] dynorphin binding. Thus in the guinea pig

brain. a kappa receptor rich tissue, dynorphin strongly prefers kappa sites. In rat brain there are substantially fewer kappa sites available; dynorphin labels these sites but also labels mu sites. That it may label this mixture of sites in rat with less affinity is shown by the fact that the dynorphin vs ³H Dyn IC₅₀ is 2.4 in rat and 1.2 in GP. Although morphine is substantially more potent in competing for [³H] dynorphin binding sites in rat brain than in guinea pig brain, it is still fifteen fold less potent than kappa agonists dynorphin A or UM1071 in displacing [³H] dynorphin binding. This demonstrates that even in a kappa poor tissue such as rat brain, [³H] dynorphin shows kappa selectivity, and thus binds to mu receptors only when kappa sites are not available. It also suggests that morphine has very low affinity for the kappa site.

DISCUSSION

Despite the characterization of a ligand according to its receptor selectivity in vitro, the selectivity in vivo involves both the selectivity of the ligand as well as the receptor type available on the post-synaptic receptor. Without careful mapping of the distribution of receptor types, it is often difficult to attribute the actions of a particular ligand to a particular receptor. For example, using serial sections of the same rat brains, Lewis et al (5) demonstrated that the autoradiographic distribution of [³H] naloxone binding (primarily a mu receptor label) matches the distribution of enkephalin immunofluorescence in the striatum. Similarly, despite the substantial potency of Beta-endorphin (B-END) at both mu and delta receptors in vitro, destruction of the B-END cell bodies in the arcuate nucleus of the rat produces a selective increase in delta receptors in the thalamus (6). Consequently, it is not usually possi-

Fig. 1

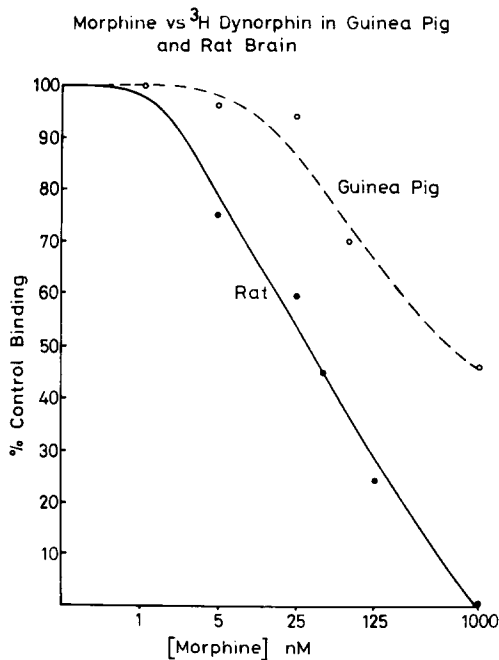
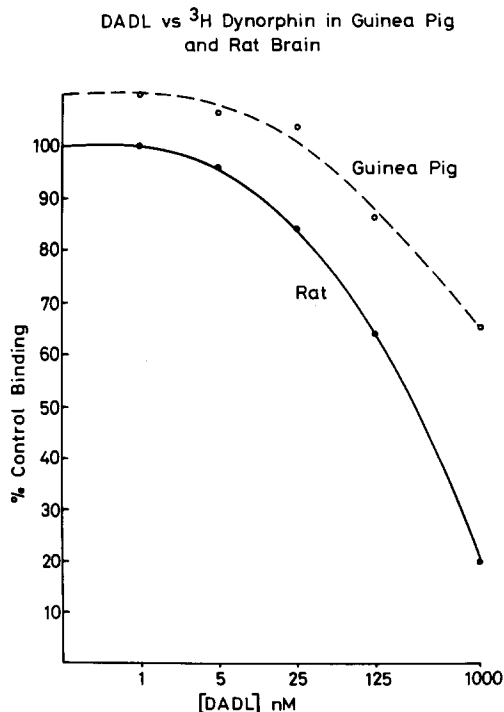


Fig. 2



ble to draw direct conclusions about the interaction of an opioid peptide with its receptor in vivo based on in vitro binding data. However, the extreme selectivity shown by dynorphin for the kappa receptor in guinea pig in vitro suggests that it interacts solely with the kappa receptor in vivo. The situation is not as clear in the rat. In this case, dynorphin appears to interact with both mu and kappa sites. Since there appear to be fewer kappa sites in rat, it is not surprising that dynorphin may synapse with mu sites as well. Consequently, there may be pathways in which dynorphin interacts with mu receptors as well as with kappa receptors. Studies comparing the autoradiographic distribution of kappa sites as well as the immunocytochemical distribution of dynorphin should resolve this issue.

ACKNOWLEDGEMENTS

This work was supported by NIDA grants DA 02265 and DA 00154 to HA and training grant # T32-MH-15794 to EY.

REFERENCES

1. C. CHAVKIN, I. JAMES and A. GOLDSTEIN, *Science* 215 413-415 (1982).
2. A. CORBETT, S.J. PATTERSON, A.T. MCKNIGHT, J. MAGNAN and H.W. KOSTERLITZ, *Nature* 299 79-81 (1982).
3. A. PFEIFFER and A. HERZ, *Bioc. Bioph. Res. Comm.* 101 38-44 (1981).
4. K.J. CHANG, E. HAZUM and P. CUATRECASAS, *Proc. Nat. Acad. Sci., USA* 78 4141-4145 (1981).
5. M.E. LEWIS, H. KHACHATURIAN and S.J. WATSON, *Life Sciences* 31 2271-2273 (1982).
6. E. YOUNG, J. OLNEY and H. AKIL, *Life Sciences* 31 1343-1346 (1982).