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Morphiceptin: Biphasic Inhibition of μ-Opiate Receptor Ligands

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VILLIGER, J. W. *Morphiceptin: Biphasic inhibition of μ-opiate receptor ligands.* PHARMACOL BIOCHEM BEHAV **21**(5) 705–706, 1984.—The interaction between morphiceptin and the morphine (μ) opiate receptor present in rat brain membranes has been examined. Detailed competitive displacement curves of morphiceptin against the μ receptor ligands [^{3}H]fentanyl and [^{3}H]naloxone were biphasic, with Hill coefficients of 0.78 and 0.60 respectively. Hoftsee plots of these displacement curves suggested that 30–35% of the morphiceptin binding was to a high affinity site and the residual binding was to a site with lower affinity. These results indicate that morphiceptin binding to the μ opiate receptor does not obey the law of mass action, and raises the possibility that morphiceptin distinguishes subclasses of μ binding site.

Morphiceptin [^{3}H]Naloxone Morphine μ Receptor [^{3}H]-Fentanyl

MORPHICEPTIN (NH_4-Tyr-Pro-Phe-Pro-$CONH_2$) is a tetrapeptide derived from β-casein which has potent opiate agonist activity [1,3]. Studies indicate that morphiceptin is selective for morphine (μ) opiate receptors, having an affinity 1000 times greater for μ than enkephalin (δ) or benzomorphan receptors [2,3]. Previously published results have suggested both monophasic [2, 3, 5] and biphasic [11] displacement curves for morphiceptin displacing μ opiate receptor ligands.

Here I have examined in greater detail the interaction between morphiceptin and the μ opiate receptor agonist [^{3}H]-fentanyl and the μ antagonist [^{3}H]naloxone.

[^{3}H]Naloxone (50 Ci/mmole) was obtained from New England Nuclear Corp., Boston. [^{3}H]Fentanyl (12.4 Ci/mmole) was kindly supplied by Janssen Pharmaceutica, Beerse, Belgium. Drugs used were morphiceptin (Sigma) and levorphanol tartrate (Roche).

The preparation of rat brain (Wistar) membranes and ligand binding assay was performed as described previously (6.7). The membranes were stored at −20°C for no more than 2 weeks. Once thawed and rehomogenized, the membranes (20 mg tissue wet weight in 2 ml of 50 mM Tris HCl buffer, pH 7.4) were incubated with 0.4 nM [^{3}H]naloxone or 2.0 nM [^{3}H]-fentanyl at 20°C for 30 min in the absence and presence of unlabelled drugs, quickly filtered through Whatman GF/B glass filters, and washed with 2×5 ml cold buffer. Nonspecific binding was determined in the presence of 1 μM levorphanol. Under these conditions, [^{3}H]naloxone and [^{3}H]fentanyl have previously been shown to primarily label μ receptors [8,9].

A curve-fitting programme (Standard-Pac, HP85 Desktop Computer) was used to determine the function that best fitted Eadie-Hoftsee plots and asymptotes were drawn in by hand. K_D values were obtained from the negative reciprocal of the slope of the asymptotes. The same programme was used to perform linear regression analysis of the Hill plots.

RESULTS

Figure 1 shows that morphiceptin displacement of 0.4 nM [^{3}H]naloxone was biphasic, with a Hill coefficient of 0.60±0.05 (n=7). Morphiceptin displacement of the μ receptor agonist [^{3}H]fentanyl (2.0 nM) was also biphasic (Fig. 2), with a Hill coefficient of 0.78±0.10 (n=5).

Eadie Hoftsee plots of these displacement curves were also curvilinear with approximately 30–35% of the binding being high affinity and the remaining morphiceptin binding being lower affinity. Preliminary estimates of the affinity constants for these sites were 5 nM for the high affinity site and 100 nM for the low affinity site.

DISCUSSION

The results of this study and another using [^{3}H]dihydromorphine [10] clearly indicate that the binding of morphiceptin to the μ-opiate receptor does not obey the law of mass action. This deviation from the law of mass action may be described by a number of molecular mechanisms, including multiple classes of opiate receptor, negative cooperativity, ternary complex formation and others [4]. While there is no evidence to rule out the latter possible interpretations, results obtained using the irreversible high affinity μ receptor antagonist naloxazone [11] raise the possibility that morphiceptin binds to multiple receptors. These authors found that pre-treatment of membranes with naloxazone blocks both high affinity [^{3}H]opiate binding [10] and the high affinity component of morphiceptin binding [11]. Should morphiceptin prove to have differential affinity (≈20 fold as suggested by the present results) for high and low affinity μ receptors, then this peptide may prove useful for future characterization of these receptor subtypes and their physiological significance.

The reason for the discrepancy between the biphasic morphiceptin displacement curves reported here and

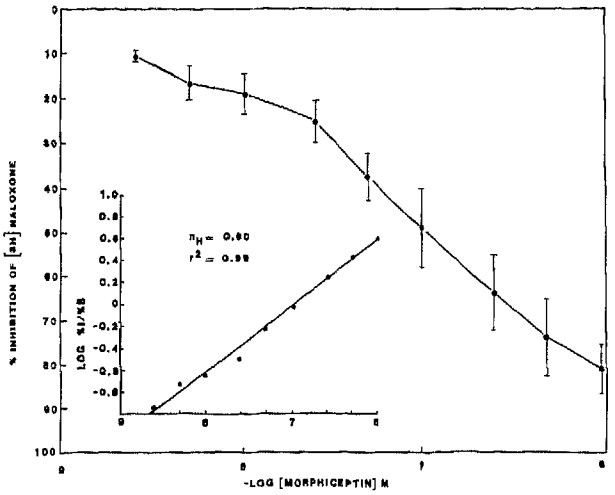

FIG. 1. Inhibition of [^{3}H]naloxone (0.4 nM) binding by morphiceptin. The curve given is the mean±standard deviation of 7 experiments. Hill plots were constructed by plotting the Log (Drug) vs. log %I/%B where %I and %B are the percentage of specifically inhibited (I) and bound (B) [^{3}H]opiate. Inset: Hill plot of this data (n_H=6.0±0.05).

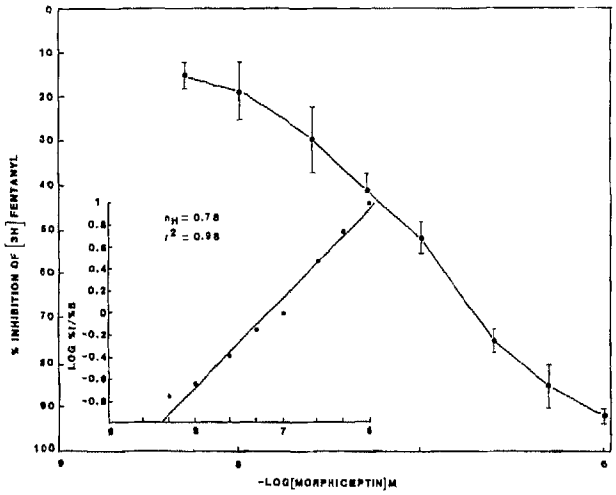

FIG. 2. Inhibition of [^{3}H]fentanyl (2.0 nM) binding by morphiceptin. The curve given is the mean±standard deviation of 5 experiments. Inset: Hill plot of this data (n_H=0.78±0.10).

elsewhere [11] and the monophasic curves previously reported [2, 3, 5] are not clear. However, the results reported by Chang *et al.* [2,3] of morphiceptin displacing [^{3}H]naloxone and [^{3}H]-dihydromorphine [2,3] were graphic and did not contain points at low morphiceptin concentrations. Thus, high affinity morphiceptin binding may have been missed in these early studies. The difference between the results reported here and those of Osborne and Herz [5] could be due to a tissue difference, since these authors studied opiate receptors in the bovine retina.

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