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KAPPA OPIATE BINDING SITES IN HUMAN PLACENTA

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SUMMARY : [^{3}H] etorphine, an agonist of μ, δ and κ receptors and [^{3}H] ethylketocyclazocine, a κ agonist, bind to one class of binding sites with a same maximum number of binding sites on human placental membranes. The pharmacological characteristics and the sensitivity to sodium ions are strictly identical for both ligands and correspond to the pharmacological properties of the κ receptor. This is also confirmed by the absence of detectable specific binding for [^{3}H]-(DAla)^2-(DLeu)^5 enkephalin, a δ agonist and [^{3}H]DHM, a μ agonist. Human placental membranes can therefore be considered as a tissue containing a homogenous population of κ opiate binding sites.

INTRODUCTION

Opiate stereospecific binding sites have been described in human placental membranes by using [^{3}H] etorphine (1).

In rat brain two types of opiate receptors have been biochemically characterized : μ and δ receptors (2,3). Morphine like drugs are more selective agonists of μ receptors whereas opioid peptides are potent agonists of δ receptors.

Morphine and enkephalins display a very low affinity for placental opiate binding sites suggesting that these binding sites do not correspond either to μ or to δ opiate receptors (4).

A recent report (5) provided strong biochemical evidences for the existence of another type of opiate receptors in guinea-pig brain which pharmacologically corresponds to the κ receptors previously described (6,7). This binding site is relatively insensitive to μ and δ drugs but it is known that etorphine and some benzomorphans such ethylketocyclazocine are potent agonists for this site (5). Since etorphine has been shown to interact with the

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same affinity with μ, δ and κ receptors (5) we determined the binding characteristics of [^{3}H] ethylketocyclazocine, a κ agonist, on human placental membranes.

MATERIALS AND METHODS

Preparation of tissue section

Human term placentae were collected on ice immediatly after vaginal delivery or cesarian section, minced with scissors and washed several times with cold Tris HCl buffer (0.05 M, pH = 7.4, 25°C). All further operations were carried out at 4° C. The tissue was freed from blood vessels and connective tissue and homogenized in 5 vol Tris HCl buffer (0.05 M, pH = 7.4) with a polytron (20 % maximal speed : 20 seconds). The homogenate was centrifuged at 1000 g for 10 minutes and the supernatant fluid was spun at 100,000 g for 30 minutes ; the resulting pellet was washed once and spun again for an additional period of 30 minutes at 100,000 g. The final pellet was homogenized and then diluted with Tris buffer (0.05 M, pH = 7.4, 25° C) to give a final protein concentration of about 1 mg/ml.

Binding assay

Placental membranes (0.8 mg protein) were incubated with [^{3}H] ligands, always in quadruplate, at 37° C for 30 minutes. Non specific binding of [^{3}H] ethylketocyclazocine was measured with 10^{-6} M of ketocyclazocine while 10^{-6} M levorphanol was used for [^{3}H] etorphine, [^{3}H] DHM and [^{3}H] $(DAla)^2$-$(Dleu)^5$ enkephalin binding. At the end of the incubation time the reaction mixtures were filtered under reduced pressure through Whatman glass Fiber disks (GF/B) and washed with 10 ml of ice cold Tris buffer (0.05 M, pH = 7.4, 25° C). The filters were dried and counted in 10 ml of toluene scintillation cocktail.

Protein concentrations were estimated by the method of Lowry and al (8).

RESULTS AND DISCUSSION

The Scatchard analysis of [^{3}H] etorphine binding to human placental membranes (fig 1 A) gives a linear plot suggesting a single class of binding sites (K_D = 0.69 ± 0.15 nM ; B_{Max} = 66 ± 13 fmoles/mg protein). However a recent report (5) indicates that this ligand binds with the same affinity to μ, δ and κ receptors.

[^{3}H] ethylketocyclazocine, a κ agonist, binds also to a single class of binding sites on placental membranes(K_D = 0.33 ± 0.07 nM) (fig 1 B) with a maximal number of binding sites (B_{Max}) (Table 1) which is not significantly different from that observed with [^{3}H] etorphine.

The relative potency of opiate and opioid peptides to displace either bound [^{3}H] etorphine or bound [^{3}H] ethylketocyclazocine is the same (Table 2). The agonists for μ receptors such as morphine, fentanyl, oxymorphone or for δ receptors such as enkephalins derivatives display a very low affinity for

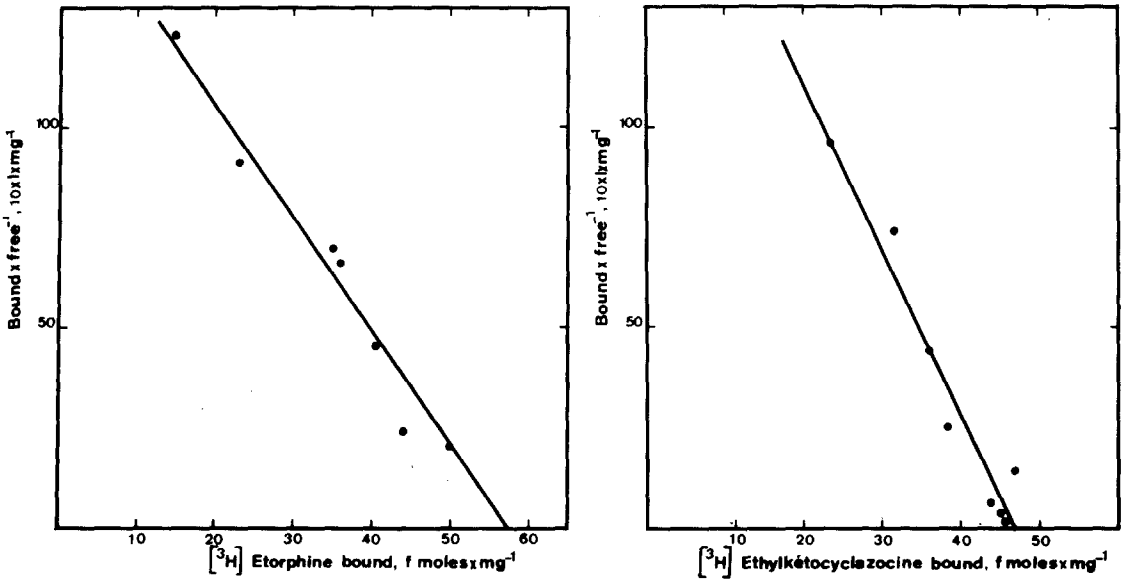

Fig. 1 : Scatchard plots of specific [^{3}H] etorphine binding (A) and specific [^{3}H] ethylketocyclazocine binding (B) to human placental membranes. Each value is the mean of quadruplate determinations. The experiment shown in representative of 5 such experiments.

[^{3}H] etorphine and [^{3}H] ethylketocyclazocine placental binding sites. In contrast benzomorphan analogs : cyclazocine, ethylketocyclazocine and pentazocine are strong inhibitors of [^{3}H] etorphine and [^{3}H] ethylketocyclazocine binding.

Table 1

Binding characteristics of [^{3}H] etorphine, [^{3}H] ethylketocyclazocine, [^{3}H] dihydromorphine and [^{3}H](DAla)^2-(DLeu)^5-enkephalin to human placental membranes.

Tritiated ligands	Equilibrium dissociation constant (nM)	Maximal number of binding sites B_{Max} (fmoles/mg protein)
Etorphine	0.69 ± 0.15 (n=(5)	66 ± 13
Ethylketocyclazocine	0.33 ± 0.07 (n=5)	54 ± 12
Dihydromorphine (0.1-5 nM)	no detectable specific binding (n=3)	
(DAla)^2-(DLeu)^5-enkephalin (1-10 nM)		

Results are expressed as mean ± s.e.m., n indicated the number of separate experiments.

Table 2

Inhibition of [^{3}H] etorphine and [^{3}H] ethylketocyclazocine binding by various opiates and opioid peptides.

Drugs	[^{3}H] ethylketocyclazocine	[^{3}H] etorphine
Cyclazocine	1	1
Etorphine	1.69 ± 0.37	1.15 ± 0.05
Ketocyclazocine	1.90 ± 0.14	1.35 ± 0.39
Naltrexone	2.53 ± 0.11	2.51 ± 0.26
Nalorphine	5.09 ± 0.77	4.53 ± 0.95
Levorphanol	12.7 ± 0.5	14.6 ± 1.0
Naloxone	15.8 ± 0.6	16.8 ± 4.2
Pentazocine	21.8 ± 2.1	19.4 ± 4.2
Fentanyl	166 ± 17	127 ± 11
Morphine	210 ± 17	204 ± 5
Oxymorphone	240 ± 23	150 ± 12
(DAla)^2-Leu^5-enkephalinamide	810 ± 84	1,900 ± 102
(DAla)^2-Met^5-enkephalinamide	1,168 ± 383	660 ± 72
(DAla)^2-(Dleu)^5-enkephalin	6,380 ± 918	5,990 ± 465
Meperidine	5,526 ± 1,098	1,220 ± 122

Binding assays were performed at 37° C for 30 minutes in the presence of [^{3}H] etorphine or [^{3}H] ethylketocyclazocine (1 nM). The IC50 values were calculated by linear regression from log-probit plots where each drug was checked at five concentrations in four separate experiments. The IC50 values are given relative to cyclazocine which were respectivaly 5.2 ± 1.15 nM for [^{3}H] ethylketocyclazocine binding and 2.6 ± 0.5 nM for [^{3}H] etorphine binding.

The sodium ions (100 mM) depress [^{3}H] etorphine and [^{3}H] ethylketocyclazocine binding but the inhibition is less important than that observed for μ receptors (9). Potassium ions are without effect between 0 and 50 mM then they slightly depresse binding of both ligands at 100 mM.

As shown in fig 2, the sodium decreases in the same manner [^{3}H] etorphine and [^{3}H] ethylketocyclazocine binding. These results clearly show that [^{3}H] etorphine and [^{3}H] ethylketocyclazocine bind to the same class of binding si-

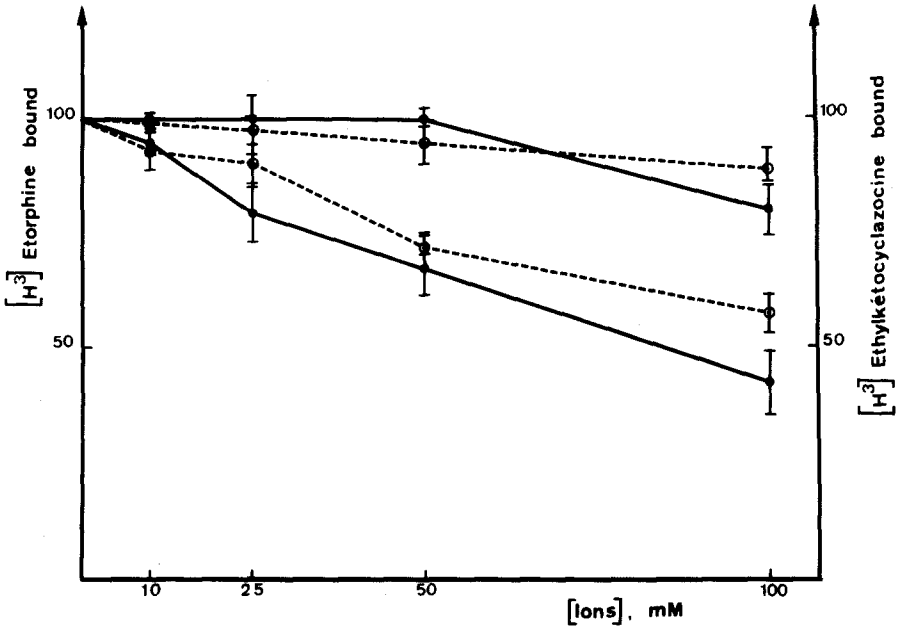

Fig. 2 : Effect of sodium and potassium ions on the stereospecific binding of [^{3}H] etorphine (————) and [^{3}H] ethylketocyclazocine (----------). Human placental membranes, suspended in TRIS-HCl buffer with or without sodium or potassium at various concentrations were incubated at 37° C for 30 min either with 1 nM [^{3}H] etorphine or with 1 nM [^{3}H] ethylketocyclazocine. Specific binding is expressed as per cent of control measured in Tris-HCl buffer. Upper curves : potassium ; lower curves : sodium ions.

tes on human placental membranes. As a matter of fact, a mixture of the two ligands gives a similar number of binding sites without any additivity. (Unpublished data).

The pharmacological characteristics of this binding and its low sensitivity to sodium ions are in good agreement with the characteristics of a κ receptor (5,9) -These results are confirmed by the absence of detectable specific binding for [^{3}H] dihydromorphine, a μ agonist, and [^{3}H] (DAla)^2-(Dleu)^5 enkephalin, a selective δ agonist, in the range of concentrations reported to achieve the saturation of respectively μ and δ opiate receptors (Table 1). The present work demonstrates that human placental membranes contain a homogenous population of opiate binding sites which has all the characteristics of kappa receptors.

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