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Opiate Receptor Binding Sites in Human Brain

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Subclasses of opiate receptor binding sites in human brain membranes were investigated by means of competitive binding techniques. The experimental data were analyzed by use of a computerized non-linear regression curve fitting program. μ-, δ- and $\varkappa$-types of opiate binding were found in 5 different regions of the brain. A more extensive analysis of the regional distribution of subclasses of opiate binding sites was performed using a simple sequential inhibition technique. This method was shown to yield results which are comparable to those obtained by computer analysis of multiple tracer displacement curves. $\varkappa$- and μ-sites represented the major component of binding in most brain areas whereas δ-sites were fewer in number. The 3 types of binding showed different distribution patterns, suggesting that they are independent from each other. The distribution pattern observed in human brain resembled the one observed in rat brain, although $\varkappa$-sites appear to represent a more important, and δ-sites appear to represent a less important, fraction of binding in human as compared to rat brain.

INTRODUCTION

The concept of multiple opiate receptors was developed by Martin[13] based upon the observation that various benzomorphans which antagonized morphine actions produced analgesia themselves. These compounds were thought to act at $\varkappa$-opiate receptors which were distinct from μ-receptors at which morphine was assumed to act. In contrast to morphine, $\varkappa$-agonists appeared to display a low addiction potential as they did not cause drug-seeking behaviour after prolonged exposure[13]. Their clinical use was, however, hampered by the frequent occurrence of psychotomimetic side effects[9,13] ascribed to an interaction with a third type of receptor termed σ, at which the N-allyl substituted benzomorphan SKF 10,047 was suggested to act as a prototype ligand[14].

The discovery of the short-chain endogenous opiate peptides, the enkephalins, permitted the distinction of enkephalin or δ-receptors from μ- or morphine receptors by both smooth muscle bioassays and radioligand binding studies[11]. Recently, binding sites displaying high affinity for benzomorphans and oripavines but low affinities for dihydromorphine and enkephalins have been differentiated in rat and guinea pig brain by the use of radioreceptor assays[2,10,16]. These binding sites could represent $\varkappa$-binding sites. No clear evidence was found indicating the presence of a binding component displaying preference for the σ-agonist SKF 10,047[16].

Obviously, there is considerable interest in acquiring information as to the different types of opiate receptor sites present in human brain. This would moreover permit the selection of laboratory animals, the receptor binding characteristics of which resemble the ones observed in human brain. In a recent study, Bonnet et al.[1] provided qualitative evidence for the occurrence of μ- and δ-sites in human brain. The aim of the present study was to quantitatively

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investigate the existence of μ-, δ-, $\varkappa$- and σ-opiate binding sites in human brain. Competitive radioligand binding techniques were used in conjunction with computerized evaluation of the data[15] as previously employed in rat brain[16,17]. Moreover, the regional distribution of subtypes of opiate binding sites was investigated.

MATERIALS AND METHODS

Subjects. Brains were obtained from 5 subjects, a 16-year-old girl who died from acute alcohol intoxication, a 66-year-old woman and from 3 men (33–45-years-old) who died from acute heart attacks. The brains were removed within 3.5–5.5 h after death and frozen at —70 °C. Further details regarding the dissection procedure were described by Gramsch et al.[6].

Binding experiments. Brain tissue was thawed and homogenized with an Ultraturrax in 30 volumes of Tris buffer (pH 7.4, 50 mM) and centrifuged at 40,000 *g* for 20 min. The supernatant was discarded and the pellet was resuspendend in the buffer and centrifuged as before. For binding assays the tissue was suspended in 100 or 200 (original tissue weight: volume) volumes of the assay buffer. Tris Krebs–Ringer buffer of the following composition was used (in mM): NaCl, 118; KCl,4.75; $MgSO_4$, 1.2; $CaCl_2$, 2.54; Tris, 50; the pH was adjusted to 7.4.

Tritiated ligands were incubated (0.1–0.5 nM) with the homogenate and the unlabeled ligands for 40 min at 35 °C in polypropylene tubes (Baker, Gross Gerau, Germany). Competition curves were constructed by the addition of increasing concentrations of unlabeled ligand. Duplicate tubes were run at each concentration. Separation of bound- from free-ligand was performed by the rapid filtration technique as previously described[16].

Data analysis. The least squares non-linear regression curve fitting program used has been described by Munson and Rodbard[15]. It is based on the law of mass action and provides parameter estimates ($\pm$S.E.) of binding capacities and affinities (K_d) which fit best to the experimental data points. Models assuming one, two or three binding components can be compared. The most appropriate model of binding is chosen statistically by an *F*-test, comparing the goodness of fit of the curves with the data points. The program also permits the simultaneous analysis of multiple curves obtained with various combinations of labeled and unlabeled ligands. This approach usually permits a more precise estimation of binding parameters[17].

Tables. The *F*-values given in the tables indicate the statistical improvement in goodness of fit achieved by the more complex model, assuming an additional binding site. The degrees of freedom (df = number of data points minus number of parameters) are indicated for the more complex models.

Materials. Tritiated ligands, dihydromorphine (73.2 Ci/mmol), diprenorphine (9.1 Ci/mmol) and (D-Ala^2,D-Leu^5)enkephalin (DADL) were obtained from Amersham Buchler, Braunschweig, Germany. SKF 10,047 (42 Ci/mmol) and ethylketocyclazocine (15 Ci/mmol) were purchased from New England Nuclear, Dreieich, Germany. Unlabeled substances, ethylketocyclazocine (Sterling Wintrop, Rensselaer, U.S.A.) SKF 10,047 (Addiction Res. Center, Lexington, KY), DADL (Bachem, Bubendorf, Switzerland), (D-Ala^2,MePhe^4)enkephalin, (H-Tyr-D-Ala-Gly-MePhe-NH-CH_2-CH_2-OH) (DAGO) (Code Number RX 783006) and bremazocine were gifts from Dr. Römer, (Sandoz, Basel, Switzerland). Tritiated substances were routinely purified by reverse phase HPLC except for diprenorphine which was 95% pure as tested by TLC.

RESULTS

A characterization of the types of opiate binding sites occurring in human brain was performed in frontal cortex membranes. The prototype ligands at μ-, δ-, σ- and $\varkappa$-sites (i.e. dihydromorphine, (D-Ala^2,D-Leu^5)enkephalin (DADL), SKF 10,047 and ethylketocyclazocine)[11,14] were used as labeled ligands and each one was displaced by the same 4 unlabeled opiates.

[^{3}H]dihydromorphine. [^{3}H]dihydromorphine labeled 2.2 $\pm$ 0.7 pmol/g tissue of binding sites with an apparent equilibrium dissociation constant of 0.7 $\pm$ 0.3 nM (K_d). All of the opiate ligands tested displaced [^{3}H]dihydromorphine with a high affinity (Table I). Furthermore, each of the competition curves was well fitted by a single site model; the fits were not improved by a two site model, a result consistent with the notion that dihydromorphine was interacting exclusively with μ-receptor sites.

TABLE I

Apparent K_d values of opiates for [^{3}H]dihydromorphine and [^{3}H](D-Ala^2,D-Leu^5)enkephalin binding sites

The reported estimates of K_d values ± S.E. were obtained by simultaneous computerized curve fitting of 2–4 displacement experiments against either [^{3}H]dihydromorphine or [^{3}H](D-Ala^2,D-Leu^5)enkephalin. The labeled ligands were assumed to interact with a single binding component, since the assumption of two binding sites did not improve the goodness of fit significantly.

Ligand	*[^{3}H]dihydromorphine K_d (nM)*	*[^{3}H](D-Ala,D-Leu) enkephalin K_d (nM)*
Dihydromorphine	0.67 ± 0.3	—*
(D-Ala,D-Leu)enkephalin	3.4 ± 1.0	1.28 ± 0.2
(D-Ala,MePhe)enkephalin	1.3 ± 0.8	—*
Ethylketocyclazocine	0.6 ± 0.3	2.9 ± 2.5
SKF 10,047	2.2 ± 0.2	18.2 ± 1.8
Bremazocine	0.3 ± 0.05	0.6 ± 0.07
Diprenorphine	0.15 ± 0.13	0.29 ± 0.11

* Values for these ligands are presented in Table II, since the assumption of two distinct binding components significantly improved fitness.

[^{3}H]DADL. The maximal number of binding sites for [^{3}H]DADL varied between 1.6–6.3 pmol/g tissue (3.8 ± 2.3) in 4 subjects, and the apparent K_d was 1.3 ± 0.2 nM. Various $\varkappa$-ligands, SKF 10,047 and diprenorphine displaced [^{3}H]DADL from a single apparent binding component (Table I). In contrast, the competition curves of the μ-ligands dihydromorphine and (D-Ala^2,MePhe^4)enkephalin (DAGO) (Fig. 1) were more complex, being flat and biphasic respectively. These data were best described by a model involving 2 classes of binding sites (Table II). The apparent equilibrium dissociation constants

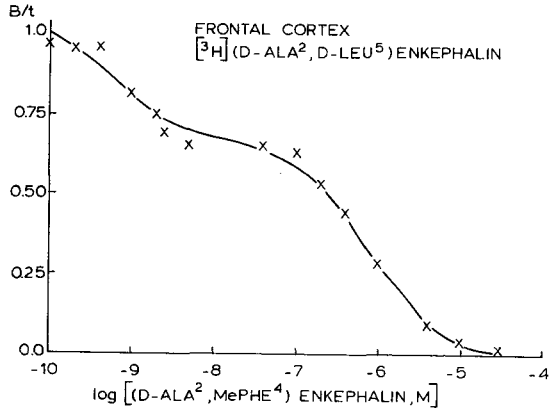

Fig. 1. Inhibition of [^{3}H](D-Ala^2,D-Leu^5)enkephalin binding by (D-Ala^2,MePhe^4)enkephalin in human frontal cortex. The curve was drawn by computer modelling of the experimentally determined data. The fraction of specifically bound [^{3}H](D-Ala^2,D-Leu^5)enkephalin is plotted on the ordinate and the abscissa shows the total ligand concentration. The data are from one representative experiment out of the 3 presented in Table II.

of dihydromorphine and DAGO for the 2 sites differed by 2 and 3 orders of magnitude respectively. The high affinity component of the μ-ligands (R_1 in Table II) corresponds well to the μ-site identified with [^{3}H]dihydromorphine (Table I). The low affinity component of the μ-ligands (R_2 in Table II) had high affinity for DADL and thus shows the characteristic affinity pattern of a δ-site[11,17].

[^{3}H]ethylketocyclazocine and [^{3}H]SKF 10,047. Unlabeled ethylketocyclazocine and SKF 10,047 both displaced [^{3}H]ethylketocyclazocine and [^{3}H]-SKF 10,047 completely from one apparent binding component (Fig. 2). The maximal binding capacity was 8.1 ± 0.8 pmol/g frontal cortex tissue (9 curves fittde simultaneously, performed with tissue from 3 subjects including homologous and heterologous combinations of the 2 ligands) and the apparent equilibrium dissociation constant was 0.9 ± 0.1 for ethylketocyclazocine and 2.3 ± 0.4 for SKF 10,047. Competition curves of DADL and dihydromorphine were flat and biphasic (Fig. 2). At a concentration of 10 μM dihydromorphine displaced 90–100% and DADL displaced 65–80% of the specific binding (Fig. 2). The curves modeled best to 2 binding sites to which the affinities of dihydromorphine and DADL differed by more than 2 and 3 orders of magnitude, respectively (Table III).

The binding component displaying high affinity for ethylketocyclazocine and SKF 10,047 but low affinity for dihydromorphine and DADL (R_2 in Table III) shows the characteristic affinity pattern of $\varkappa$-

TABLE II

Parameter estimates of binding capacity and K_d for (D-Ala², MePhe⁴)enkephalin and dihydromorphine obtained from computer modelling of competition curves with [³H](D-Ala,D-Leu)enkephalin in frontal cortex

Three curves were fitted simultaneously. The K_d value of [³H](D-Ala²,D-LEU⁵)enkephalin to R_1 and R_2 was assumed to be 1.2 nM. The df were 29. $P < 0.01$ for comparison of the 2-site with the 1-site model. It should be noted that the experiments using dihydromorphine and (D-Ala,MePhe)enkephalin were performed with tissue from different subjects.

	R_1		R_2		F
	capacity (pmol/g)	*K_d (nM)*	*capacity (pmol/g)*	*K_d (nM)*	
(D-Ala²,MePhe⁴)enkephalin	2.3 ± 0.3	0.55 ± 0.3	3.9 ± 0.2	585 ± 81	36.8
Dihydromorphine	2.8 ± 0.3	1.4 ± 0.8	2.0 ± 0.2	251 ± 109	22.9

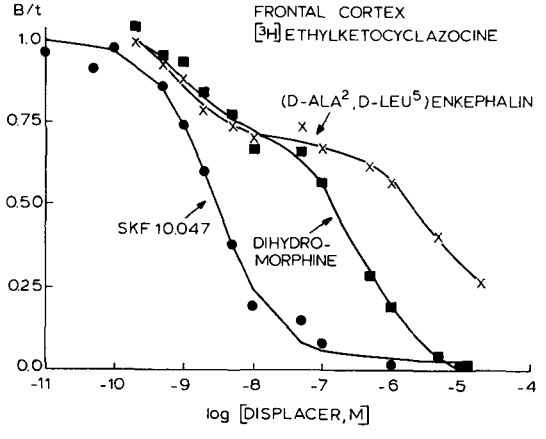

Fig. 2. Inhibition of [³H]ethylketocyclazocine binding to human frontal cortex membranes by (D-Ala²,D-Leu⁵)enkephalin, dihydromorphine and SKF 10,047.

sites described in rat brain[17]. The high affinity component apparent with DADL and dihydromorphine (R_1 in Table III) had a capacity of 3–4 pmol/g tissue with both ligands and may correspond to a μ-site. Unexpectedly, no δ-type binding was apparent although the data shown in Table II (which were constructed with frontal cortex tissue from the same subjects as the ones shown in Table III) were evidential of 2–4 pmol/g tissue of δ-sites. This result raised the question whether δ-sites were present in addition to μ-sites or could only be demonstrated using [³H]DADL. If δ-sites are present in addition to μ- and $\varkappa$-sites, an opiate ligand displaying similar affinities to the 3 types of opiate binding sites should permit their demonstration. Since ethylketocyclazocine and SKF 10,047 had a somewhat lower

TABLE III

Parameter estimates of binding capacity and K_d for dihydromorphine and (D-Ala², D-Leu⁵)enkephalin obtained from computer modelling of competition curves with [³H]ethylketocyclazocine ($n = 3$) and [³H]SKF 10,047 ($n = 2$) in human frontal cortex

Three and 2 curves were fitted simultaneously. The affinities of [³H]ethylketocyclazocine and [³H]SKF 10,047 were 0.9 and 2.3 nM (K_d). The df were 30 and 19 with [³H]ethylketocyclazocine and [³H]SKF 10,047, $P < 0.01$ for comparison of the 2-site with the 1-site model, respectively.

Ligand	R_1		R_2		F
	capacity (pmol/g)	*K_d*	*capacity (pmol/g)*	*K_d*	
[³H]ethylketocyclazocine					
dihydromorphine	4.1 ± 0.4	1.0 ± 0.4	5.7 ± 0.3	265 ± 40	55
(D-Ala,D-Leu)enkephalin	3.4 ± 0.3	3.4 ± 1.2	6.1 ± 0.4	7240 ± 1350	72
[³H]SKF 10,047					
dihydromorphine	4.2 ± 0.6	0.8 ± 0.5	5.3 ± 0.3	131 ± 23	27
(D-Ala,D-Leu)enkephalin	4.1 ± 1.1	1.2 ± 1.8	5.4 ± 0.5	1310 ± 709	11.6

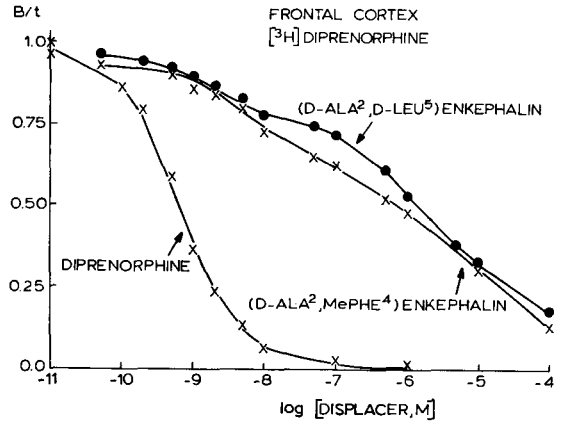

Fig. 3. Inhibition of [^{3}H]diprenorphine binding by(D-Ala^2,D-Leu^5)enkephalin, (D-Ala^2,MePhe^4)enkephalin and diprenorphine in human frontal cortex.

affinity to δ- than to μ- and $\varkappa$-sites, further experiments were performed using the non-selective opiate antagonist [^{3}H]diprenorphine.

[^{3}H]diprenorphine. [^{3}H]diprenorphine labeled a homogenous population of binding sites having a maximal binding capacity of 14.8 $\pm$ 0.9 pmol/g of frontal cortex tissue and an apparent equilibrium dissociation constant of 0.22 $\pm$ 0.03 nM (3 curves). This K_d value was in agreement with the apparent affinities estimated for diprenorphine from competition curves with [^{3}H]dihydromorphine and [^{3}H]-DADL (Table I) and therefore suggests that diprenorphine displayed about equal affinities for δ- and μ-binding sites.

The competition curve of ethylketocyclazocine against [^{3}H]diprenorphine in frontal cortex modeled to 2 apparent binding components, one of 10 $\pm$ 1.2 and a second of 6 $\pm$ 1.3 pmol/g tissue. The affinity of ethylketocyclazocine was 0.7 $\pm$ 0.3 and 13.5 $\pm$ 3.9 nM (K_d), respectively (2 curves, df = 19, F = 103, P <0.01). The capacity of the high affinity component was in agreement with the respective component identified using [^{3}H]ethylketocyclazocine (Table III). The low affinity component probably corresponds to a second type of $\varkappa$-binding site described in detail in a separate publication[18]. However, δ-type binding might also contribute to the low affinity component.

Competition curves of DADL and of DAGO against [^{3}H]diprenorphine were multiphasic and very flat (Fig. 3). Due to the complex nature of the curves, binding capacities and affinities of the ligands could not be estimated accurately from displacement curves against [^{3}H]diprenorphine. The experimental data for both ligands, however, modeled best to 3 apparent binding components (Table IV). A better definition of the binding parameters would have required the simultaneous modeling of displacement curves obtained with DADL and DAGO against [^{3}H]diprenorphine and [^{3}H]DADL. This would have permitted a better description of the interaction with δ- and μ-sites. The capacity of apparent δ-sites as estimated with [^{3}H]DADL was usually greater than when estimated with [^{3}H]diprenorphine. Curves obtained with these two tritiated

TABLE IV

Parameter estimates of binding capacity and Ka for (D-Ala^2,D-Leu^5) enkephalin and (D-Ala^2,MePhe^4)enkephalin obtained from computer modelling of competition curves with [^{3}H]diprenorphine in frontal cortex

Three curves obtained either with (D-Ala^2,D-Leu^5)enkephalin or with (D-Ala^2,MePhe^4)enkephalin were analyzed simultaneously by computer fitting. R_1, R_2 and R_3 are used to indicate the binding components which were distinguished, and do not indicate similar sites for the two ligands. The affinity of tritiated diprenorphine was 0.2 nM (K_d). The df were 32. P values are indicated for statistical comparison of the 3-site with the 2-site model. Two of the subjects evaluated were different from those used for the data in Table II and III.

Displacer	R_1		R_2		R_3		F
	Capacity (pmol/g)	K_d	*Capacity (pmol/g)*	K_d	*Capacity (pmol/g)*	K_d	
(D-Ala^2,D-Leu^5) enkephalin	3.4 $\pm$ 1.2	0.3 $\pm$ 0.3	6.8 $\pm$ 1.1	42 $\pm$ 27	6.4 $\pm$ 0.5	6710 $\pm$ 2190	4.1 $P < 0.05$
(D-Ala^2,MePhe^4) enkephalin	3.7 $\pm$ 0.2	1.1 $\pm$ 0.9	6.0 $\pm$ 2.4	163 $\pm$ 173	6.0 $\pm$ 2.8	5420 $\pm$ 4050	34 $P < 0.05$

ligands were therefore not compatible and simultaneous fitting could not be achieved with reasonable results.

Further experiments were performed in order to ascertain whether DADL interacted with the δ-binding site in addition to μ-sites. Competition experiments of DADL with [^{3}H]diprenorphine were thus conducted in the presence of 100 nM DAGO. At this concentration, DAGO should be expected to saturate μ-sites while δ-sites should be occupied only to a negligible extent (assuming apparent K_d values of 1 and 500 nM for μ- and for δ-sites respectively) (Tables I and II). Under these conditions displacement curves of DADL against [^{3}H]diprenorphine modeled best to 2 apparent binding components (Fig. 4). In the frontal cortex and in the area striata of the visual cortex the apparent capacity of the δ-site was 1.5 $\pm$ 0.2 and 2.2 $\pm$ 0.6 pmol/g tissue and the K_d values were estimated to be 2.6 $\pm$ 2.0 and 1.1 $\pm$ 0.3 nM respectively (2 curves in tissue from 2 subjects each, df = 18, F = 159 for the area striata and F = 23 in the frontal cortex, $P<0.01$). This capacity of the δ-site was in agreement with the values presented in Table V for the area striata. In the frontal cortex the apparent capacity was 2-fold lower than would be expected from the displacement curves of [^{3}H]DADL by the μ-ligands (Table II) in which binding of [^{3}H]DADL to μ-sites is corrected for.

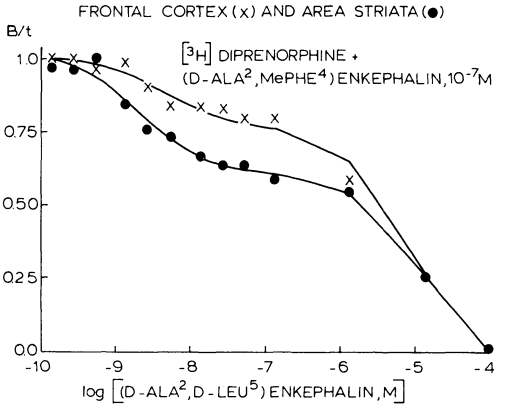

Fig. 4. Inhibition of [^{3}H]diprenorphine in the presence of 100 nM (D-Ala^2,MePhe^4)enkephalin by (D-Ala^2,D-Leu^5)enkephalin in human frontal cortex and area striata of the visual cortex.

TABLE V

Parameter estimates of binding capacity and K_d for dihydromorphine; (D-Ala^2,MePhe^4)enkephalin and (D-Ala^2,D-Leu^5)enkephalin obtained from computer modelling of competition curves obtained with multiple ligand combinations in various areas of the brain

Four to 10 curves from 2–3 different subjects were evaluated simultaneously using combinations of the 3 ligands indicated with tritiated diprenorphine, dihydromorphine and (D-Ala^2,D-Leu^5)enkephalin. The affinity of diprenorphine was 0.27 $\pm$ 0.07 in the thalamus and was 0.16 $\pm$ 0.02 nM (K_d) in the caudate nucleus. In the other structures the K_d of diprenorphine was assumed to be 0.2 nM, as no curves of [^{3}H]diprenorphine against unlabelled diprenorphine were included.
The statistical parameters were: A, df = 38, F = 42.6, n = 4; B, df = 45, F = 65, n = 8; C, df = 115, F = 26, n = 10; D, df = 63, F = 0.9, n = 7. The F-values compare the 3-site to the 2-site model. $P < 0.01$ in A–C, and not significant in D.

Proposed binding-site	*Binding capacity (pmol/g tissue)*	*Apparent affinity (K_d, nM)*		
		Dihydromorphine	*(D-Ala^2,D-Leu^5) enkephalin*	*(D-Ala^2,MePhe^4) enkephalin*
Gyrus cinguli (A)				
μ	7.2 $\pm$ 0.9	0.85 $\pm$ 0.31	9.1 $\pm$ 1.7	—
δ	2.0 $\pm$ 0.4	93 $\pm$ 12	0.67 $\pm$ 0.2	—
$\varkappa$	10.4 $\pm$ 1.5	183 $\pm$ 22	5080 $\pm$ 697	—
Nucleus caudatus (B)				
μ	5.4 $\pm$ 0.5	3.8 $\pm$ 1.6	8.1 $\pm$ 1.4	8.7 $\pm$ 2.9
δ	1.2 $\pm$ 0.2	84 $\pm$ 47	0.24 $\pm$ 0.23	175 $\pm$ 99
$\varkappa$	3.5 $\pm$ 0.3	474 $\pm$ 93	5620 $\pm$ 856	3080 $\pm$ 605
Area striata of the visual cortex (C)				
μ	1.0 $\pm$ 0.2	13.7 $\pm$ 2.7	28.6 $\pm$ 20	0.58 $\pm$ 0.5
δ	2.0 $\pm$ 0.4	476 $\pm$ 102	1.0 $\pm$ 0.33	758 $\pm$ 139
$\varkappa$	3.3 $\pm$ 0.3	200 $\pm$ 73	570 $\pm$ 2330	10700 $\pm$ 3780
Tahlamus (D)				
μ	8.9 $\pm$ 1.8	3.1 $\pm$ 0.5	3.5 $\pm$ 0.8	
$\varkappa$	5.1 $\pm$ 1.1	112 $\pm$ 23	1720 $\pm$ 421	

Regional distribution pattern of multiple opiate binding sites

The total binding capacity of [^{3}H]diprenorphine varied in membranes prepared from different brain areas. Membrane preparations from the caudate nucleus, the gyrus cinguli and the area striata of the visual cortex contained a greater capacity of high affinity sites for DADL than for the μ-ligands as assessed by displacement of [^{3}H]diprenorphine. This agrees with the high affinity of DADL to δ- and μ-sites. The capacity of the δ-site as assessed by competition with [^{3}H]DADL was moreover in approximate agreement with the δ-site apparent with [^{3}H]-diprenorphine. Simultaneous modeling of data obtained with [^{3}H]dihydromorphine, [^{3}H]DADL and [^{3}H]diprenorphine was possible and indicated, that the binding capacities and affinities of the ligands to the various sites were in agreement irrespective of the labeled ligand used. The interaction of the ligands with membranes from the area striata, the caudate nucleus and the gyrus cinguli was best described by models involving 3 different classes of binding sites (Table V). The affinity patterns of the ligands to the binding sites corresponded to μ-, δ- and $\varkappa$-sites. In the thalamus, dihydromorphine displaced [^{3}H]DADL from one apparent binding component with high affinity. In this brain area, only μ and $\varkappa$-type binding was evident (Table V). The affinities of the ligands to the same sites varied by approximately 10-fold in the different brain regions. Whether these differences indicate subtle regional variations of opiate receptor subsites or represent differences between individuals will require a study including more subjects.

An attempt was made to obtain estimates of the relative amounts of μ-, δ- and $\varkappa$-sites in various brain areas by a simple sequential displacement method. The binding of [^{3}H]diprenorphine was measured in the absence of other opiates and in the presence of 100 nM DAGO, 100 nM DAGO plus 1 μM DADL and in the presence of 100 nM diprenorphine; non-specific binding was, thus, determined. The fraction which was displaced by DAGO was taken as μ-type binding. The further displacement achieved by the addition of 1 μM DADL was assumed to represent δ-type binding, and the remaining specific binding was taken as $\varkappa$-type binding. As shown in Table VI values obtained by this sequential displacement method were in approximate agreement with binding capacities estimated by computer analysis in the thalamus, the gyrus cinguli, the nucleus caudatus and the area striata (Table V). Fig. 5, moreover, gives an illustration of the differential distribution of μ- and $\varkappa$-sites as assessed by the displacement of [^{3}H]diprenorphine in various areas of the brain.

DISCUSSION

The present study examines the interaction of opiate ligands with binding sites in the human brain. Heterologous displacement experiments were performed with combinations of radiolabeled and unlabeled putative prototype opiate ligands at μ-, δ, σ- and $\varkappa$-sites. The estimates of relative affinities thus obtained for the opiates were consistent with the concept of μ-, δ- and $\varkappa$-sites.

Dihydromorphine and DAGO interacted with a single binding component with high affinity (Table I), thereby allowing for its identification as a μ-site[10,11]. [^{3}H]DADL labeled a binding component with high affinity to which the μ-ligands displayed low affinity (Table II). DADL also had a high affinity to μ-sites. Such binding characteristics correspond to the ones of μ- and δ-sites identified in rat[2,16,17] and guinea pig brain[10,11]. The benzomorphans ethylketocyclazocine and SKF 10,047 bound to μ-sites with approximately 5-fold higher affinity than to δ-sites (Table I). [^{3}H]ethylketocyclazocine, [^{3}H]SKF 10,047 and [^{3}H]diprenorphine labeled an additional binding component with high affinity (0.2–2.3 nM) (Tables III, IV and V). To this site dihydromorphine had low affinity (100–400 nM K_d) and the enkephalin derivatives DAGO and DADL had a very low affinity of approximately 3–8 μM K_d (Tables III, IV and V). This binding site probably corresponds to $\varkappa$-sites described in the rat[2,16,17] and guinea pig brain[10]. Both, ethylketocyclazocine and SKF 10,047 had a similar affinity to $\varkappa$- and μ-sites. This observation distinguishes $\varkappa$-sites in the human from those in the rat brain. In the rat brain, ethylketocyclazocine and SKF 10,047 display approximately 3–5-fold lower affinity to $\varkappa$-, than to μ-sites[2,16,17].

In the area striata of the visual cortex, the gyrus cinguli and the nucleus caudatus, μ-, δ- and $\varkappa$-sites

TABLE VI

Distribution of opiate binding sites in various brain areas

Estimation of μ-, δ- and $\varkappa$-sites by sequential displacement of [^{3}H]diprenorphine by 100 nM (D-Ala^2,MePhe^4)enkephalin (μ-sites) 100 nM (D-Ala^2,MePhe^4)enkephalin + 1 μM (D-Ala^2,D-Leu^5)enkephalin (δ-sites) or 100 nM diprenorphine ($\varkappa$-sites). μ-, δ- and $\varkappa$-sites are expressed as percent of the total bound. The mean S.E. for subsite determination was 7.5 ± 1.2% for μ-, 4.4 ± 0.4% for δ- and 8.1 ± 1.3% for $\varkappa$-sites of the total bound. n = number of different subjects evaluated.

	Total bound pmol/g tissue	μ %	δ %	$\varkappa$ %	*n*
Frontal cortex	7.3 ± 1.3	34	25	41	3
Parietal cortex	7.3 ± 0.6	45	20	35	2
Insula	9.6	34	24	42	1
Area striata	4.3 ± 0.8	18	38	44	5
Gyrus cinguli	7.9 ± 2.3	42	19	39	4
Putamen	6.3 ± 0.6	35	36	29	2
Pallidus	4.7	38	24	38	1
Nucleus caudatus	8.7 ± 0.1	35	25	40	2
Nucleus ruber	1.5 ± 0.2	50	21	29	2
Thalamus	7.9 ± 1.5	67	9	24	3
Hypothalamus ant.	8.3 ± 2.9	45	6	49	3
Hypothalamus post.	4.7 ± 1.0	49	10	41	5
Hippocampus	3.3 ± 0.4	27	19	54	3
Septum	3.0 ± 0.3	48	18	34	2
Periaquaeductal grey	5.2 ± 1.4	46	13	41	3
Amygdala med.	12.2 ± 1.2	29	13	58	2
Amygdala lat.	11.4 ± 1.7	30	8	62	4
Substantia nigra	3.3 ± 0.8	28	6	66	3
Med. oblong. dors.	3.8 ± 0.7	42	18	40	3
Pons dors. with locus coeruleus	3.3 ± 0.1	47	17	36	3
Pons ventralis	1.5 ± 0.1	8	48	44	2
Area postrema	1.6	39	16	45	(3)*
Nucleus dentatus	0.9	16	18	66	1
Oliva inferior	0.9 ± 0.1	53	12	35	2
Tractus olfactorius	6.7 ± 0.1	27	15	57	2
Mammillary bodies	2.8 ± 0.3	46	20	34	2

* The area postrema value was obtained with tissue which was pooled from 3 subjects.

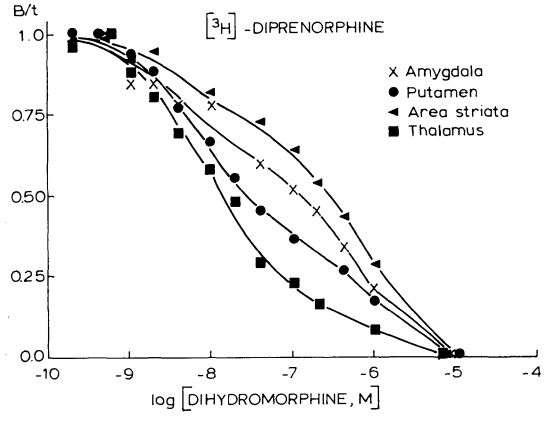

Fig. 5. Inhibition of [^{3}H]diprenorphine by dihydromorphine in various brain areas.

were shown by simultaneous computerized modeling of displacement curves obtained by the use of tritiated and unlabeled DADL, dihydromorphine and diprenorphine (Table V).

The results from these experiments indicated that the binding capacities and affinities of the 3 differentiated binding sites were in good agreement irrespective of the labeled ligand used. An exception was made by frontal cortex membranes. In this tissue, 2–4 pmol/g of δ-sites were estimated by displacement of [^{3}H]DADL with the μ-ligands (to correct for binding of DADL to μ-sites) (Table II). δ-sites could not be detected by displacement of [^{3}H]ethylketocyclazocine or [^{3}H]SKF 10,047 with μ- and δ-ligands (Table III) although the benzomorphans

completely displaced [^{3}H]DADL at rather low concentrations (Table I). The use of [^{3}H]diprenorphine permitted the demonstration of apparent δ-sites. Their binding capacity, however, was 2-fold lower than when measured by the use of [^{3}H]DADL. The possibility therefore arises, that δ-sites in the frontal cortex do not exist independently from μ-sites. The result could be explained by a model assuming topographically distinct, but allosterically related binding sites for DADL and the μ-ligands on either a single or different receptor molecules. However, the small number of subjects investigated imposes caution in the interpretation of the results.

The distribution of the 3 types of opiate binding sites was investigated by the use of a simple sequential displacement method. The non-selective opiate ligand [^{3}H]diprenorphine was displaced by saturating concentrations of the μ- and δ-ligands DAGO and DADL. This method yielded results comparable to those obtained by a more extensive analysis of multiple tracer displacement curves (Tables V and VI). A comparable approach has also been employed by Chang et al.[2], who used β-casomorphin-4-amide as a μ-ligand. The different types of opiate binding were unevenly distributed and there was no obvious correlation between the subtypes (Table VI).

μ-Type binding was highest in the thalamus, the hypothalamus, cortical structures and the cingulate gyrus. In the ventral pons, apparent μ-binding was very low whereas δ-sites accounted for almost half of the binding capacity present. In most cortical structures, especially the area striata of the visual cortex and in the extrapyramidal structures, δ-sites accounted for 20–40% of the total binding capacity and comprised less than 20% in most of the deeper brain structures. κ-Sites represented 25–65% of the total binding capacity present in all brain regions and were highest in the amygdala, the hypothalamus, the cingulate and in the cortex. The lowest relative levels occurred in extrapyramidal structures with the exception of the caudate nuclaus.

Experiments performed with dynorphin$_{1-13}$ and dynorphin$_{1-17}$[4,5,20] revealed that these peptides interacted with a very low apparent equilibrium dissociation constant of approximately 0.1 nM (K_d) with about 50% of the κ-binding component of [^{3}H]diprenorphine, whereas the affinity of β-endorphin was approximately 300-fold lower[18]. There is some correlation between the distribution of κ-sites and immunoreactive dynorphin$_{1-13}$ (ir-dynorphin) present in human brain[7]. For example, the amygdala, the caudate nucleus and the hypothalamus contain the highest levels of both κ-sites and ir-dynorphin. Moreover, in the hippocampus and the substantia nigra, two further structures containing high levels of ir-dynorphin, more than 50% of the binding capacity present is of the κ-type. The highest levels of Met-enkephalin-ir[6] occurring in the extrapyramidal system correlate well with the δ-binding capacity which is also highest in these structures.

Although opiates are known to elicit a variety of effects in humans[3,12,21,23] little information is available regarding the topographical site of, opioid action and the types of receptors involved. The effects upon neurosecretion of peripherally administered opioid peptides[8,19,21] which fail to cross the blood–brain barrier, are probably mediated by the μ-class of opiate receptors in the hypothalamus. Thus, the hypothalamus contains very few δ-receptors sites while the peptide used (FK 33824) does not interact with κ-receptor sites[2].

As discussed in a related study[18], the aversive effects characteristic of most benzomorphans[9,14] and especially SKF 10,047 could be mediated by a sub-fraction of κ-sites (thus corresponding to the σ-receptor of Martin et al.[14]). Such a subclassification of κ-sites is corroborated by the over 2000-fold differences in affinity displayed by dynorphin$_{1-17}$ to the 2 components of the κ-binding site. In contrast, various benzomorphans and naloxone completely displaced κ-binding at low concentrations[18].

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