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DIFFERENT TYPES OF OPIATE AGONISTS INTERACT DISTINGUISHABLY WITH MU,DELTA AND KAPPA OPIATE BINDING SITES

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SUMMARY

The present studies were undertaken to evaluate whether different types of opiate agonists interact in a distinguishable manner with mu, delta and kappa opiate binding sites.Two approaches were employed: (a) the well known effects of metal ions on opiate agonist binding affinities of subsite selective ligands were studied at mu, delta and kappa sites in rat brain homogenates.Binding parameters were obtained by simultaneous computeranalysis of displacement curves using the prototypic ligands dihydromorphine (DHM), (D-Ala^2, D-Leu^5)enkephalin (DADL) and ethylketocyclazocine (EKC) of the mu, delta and kappa binding sites respectively.The results show, that the effects of metal ions depend not only on the binding site, but also on the ligand under investigation. (b) The interaction of the delta agonist DADL with the mu agonist DHM was investigated at mu binding sites by characterizing the type of competition occuring between the two ligands. The interaction was of the noncompetitive type. It therefore appears that the various opiate agonists either interact preferentially with different parts of a larger receptor site area or bind to topographically distinct sites on a single receptor molecule which are coupled allosterically.

RESULTS

The effects of Na^+ (100 mM), Gpp(NH)p (60 uM) (Blume, 1978) and Mn^{++} (1 mM) (Pasternak et al., 1975) on the interaction of the mu agonist DHM, the delta agonist DADL ,the kappa agonist EKC and the opiate antagonist diprenorphine with opiate receptor sites was assessed. Simultaneous analysis by nonlinear least squares curve fitting (Munson and Rodbard, 1980) of displacement curves obtained with combinations of the 4 ligands was used to distinguish binding to mu, delta and kappa sites. As shown in table 1, Na^+ and Gpp(NH)p generally decreased and Mn^{++} increased agonist binding affinities at mu and delta sites with the exception of DADL whose affinity at mu sites was not decreased by Gpp(NH)p in unwashed rat brain homogenates. The changes in affinity observed with DHM were greater than those seen with DADL or EKC. For example, in the presence of Na^+ the affinity of DHM was decreased by 7 and 17-fold at mu and delta sites respectively, whereas those of EKC and DADL were only decreased by 1.8-3.6-fold (table 1). The affinity of EKC to kappa sites was decreased by Na^+ and Gpp(NH)p whereas those of DADL and EKC showed little change. Apparently, the effects of metal ions and Gpp(NH)p on agonist affinity depended on both, the binding site and the agonist investigated. Results suggesting a similar interpretation were recently published by Chang et al.(1981) with regard to the interaction of various opiate agonists with antagonists in rat brain and neuroblastoma x glioma hybrid cell membranes.

We recently observed that Ca^{++} selectively enhances the interaction of delta agonistic enkephalins with opiate binding sites in hypotonically washed rat

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brain membranes (Pfeiffer and Herz, 1982a) at concentrations between 0.5-3 mM Ca^{++}. Binding of mu and kappa agonists was little affected under the same conditions (table 2).

TABLE 1

EFFECT OF METAL IONS AND Gpp(NH)p ON AGONIST BINDING AFFINITIES

AGONIST:	DIHYDROMORPHINE			(D-Ala^2,D-Leu^5)ENKEPHALIN			ETHYLKETOCYCLAZOCINE		
SITE:	mu	delta	kappa	mu	delta	kappa	mu	delta	kappa
control	1	1	1	1	1	1	1	1	1
Mn^{++}	0.6	0.7	1.0	0.5	0.8	1.8	0.8	0.6	1.4
Gpp(NH)p	1.8	2.2	0.8	0.8	1.6	1.2	1.9	2.4	4.2
Na^+	7.4	17	1.1	2.0	1.8	1.0	1.9	3.6	1.9
washed	0.8	1.4	0.9	0.5	0.9	1.8	0.6	0.9	1.4
control K_d(nM)	2.8	108	385	21	1.6	3160	2.5	10	0.7

Control dissociation constants (K_d)were obtained in Tris buffer (50 mM, pH 7.4) in fresh unwashed rat brain homogenate. Affinity estimates were obtained by analysis of displacement curves obtained with tritiated and unlabeled DADL, EKC, DHM and diprenorphine as described in detail elsewhere (Pfeiffer et al.,1982). The affinity of diprenorphine varied between 0.29-0.36 nM K_d under the different conditions. The relative affinities indicated above were calculated by dividing the K_d obtained under the specified condition by the control K_d indicated.

TABLE 2

EFFECT OF Ca^{++} ON THE SPECIFIC BINDING OF OPIATES

RADIOLABELED LIGAND	% OF CONTROL BINDING IN THE PRESENCE OF 3mM Ca^{++}	
[^{3}H](D-Ala^2,D-Leu^5) enkephalin	180	Rat brain membranes were washed repeatedly with 5mM Tris buffer, pH 7.4 and resuspended by use of an ultra turrax homogenizer between the washes Binding experiments were performed in 50 mM Tris, pH 7.4, 0.5 mM EGTA ± 3.5 mM Ca^{++}. The variation between the values was usually below 10% in 2-3 independent experiments. Values for (Met^5)enkephalin, (Leu^5)enkephalin and (D-Ala^2,D-Leu^5)enkephalin were significantly different from all others (p 0.05, students t-test).
[^{3}H](D-Ala^2,MePhe^4, Met^5-(O)-ol)enkephalin	98	
[^{3}H]morphine	95	
[^{3}H]naloxone	112	
[^{3}H]diprenorphine	89	
[^{3}H]ethylketocyclazocine	100	
[^{3}H]etorphine	103	

The enhanced binding of [^{3}H]DADL was mainly due to an increase in apparent binding affinity as shown in figure 1. In case that the increase in binding affinity was related to delta sites one would not expect to observe such an increase in the presence of Ca^{++} in membranes containing very few delta sites such as diencephalic membranes (Pfeiffer and Herz, 1981). However, as shown in figure 1, Ca^{++} (3 mM) enhanced [^{3}H]DADL binding affinity in both, diencephalic membranes which contain mainly mu sites and frontal cortex membranes which contain about equal quantities of mu and delta sites (Pfeiffer and Herz, 1981).

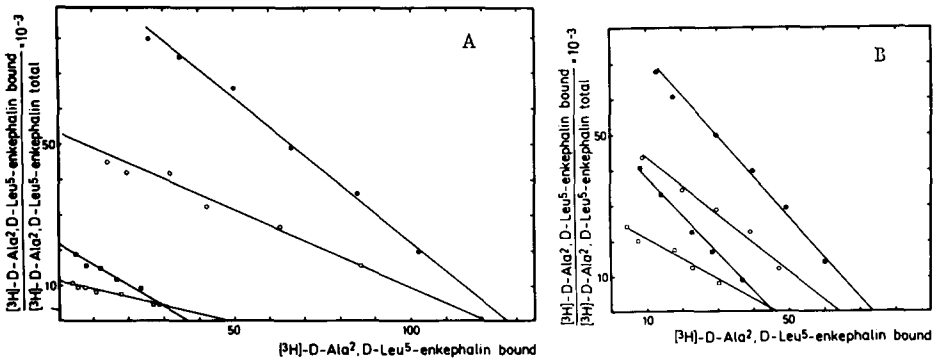

FIGURE 1

Scatchard plots of [^{3}H]DADL binding to (A) diencephalic and (B) frontal cortex membranes: Effects of Ca^{++} (3 mM) (filled symbols) and of DHM (10 nM) (squares) or DHM (10 nM) plus Ca^{++} (3mM) (filled squares)Controls are shown as open circles. The affinity of [^{3}H]DADL in frontal cortex membranes was 1.4±0.02, in diencephalic membranes 2.0±0.3 nM K_d as measured in washed membrane preparations in 50mM Tris buffer, pH 7.4 (control values).

The Ca^{++} induced increase in binding affinity was also observed in the presence of 10 nM DHM which should reduce binding of [^{3}H]DADL to mu sites in the frontal cortex and represent predominantly binding to delta sites. It would therefore appear that Ca^{++} selectively enhances binding of enkephalins with preference for delta sites to both, delta and mu sites, and that this effect is rather a characteristic of the type of agonist than of the type of binding site investigated.

These findings seemed to indicate that the various classes of opiate agonists interact in a distinguishable manner with the same opiate receptor sites. This question was therefore adressed more directly by studying the interaction of the mu ligand DHM and the delta ligand DADL at a single binding site.

According to classical Michaelis-Menton kinetics, competitive interaction of two ligands at one binding site should result in a decrease in the apparent binding affinity of the labeled ligand when measured in the presence of unlabeled competitor as compared to its absence. In the case of noncompetitive inhibition, an apparent decrease in the binding capacity of the labeled ligand should be observed in the presence of the unlabeled ligand with no change in binding affinity, as increasing concentrations of the labeled ligand would not affect the noncompetitive inhibitors binding at a distinct site. These considerations are not applicable to situations where two ligands interact with multiple binding sites with different affinities.

As shown in figure 1, [^{3}H]DADL labeled a single apparent binding component in frontal cortex membranes as indicated by the linear scatchard plot. In the presence of 10 nM unlabeled DHM, the apparent binding capacity of [^{3}H]DADL was reduced by approximately 40% with little change in affinity as indicated by the similar slopes of both lines. This indicates a noncompetitive type of interaction between the mu and the delta ligand. However, since both, mu and delta sites are present in the frontal cortex membrane preparation this may be interpreted in terms of an allosteric interaction between mu and delta sites as recently proposed by Rothman and Westfall (1982); i.e. morphine would reduce the binding capactity of delta sites via an interaction with mu sites. Alternative-

ly, DHM could inhibit the binding of [^{3}H]DADL to mu sites allosterically by interacting with a somewhat different site on the same mu receptor molecule.

In order to distinguish between these two possibilities, the same experiment as described above was repeated in diencephalic membranes in which binding of DADL and DHM occurs to mu sites predominantly. Allosteric interaction between mu and delta receptor molecules seems improbable in the presence of very few delta sites, and should at least be reduced. However, as shown in figure 1, the apparent binding capacity of [^{3}H]DADL was reduced by approximately 60% in the presence of 10 nM DHM with little change in affinity. DHM thus was more potent in diencephalic than in frontal cortex membranes. Another possible explanation would be that DHM preferentially reduced [^{3}H]DADL binding to mu sites leaving only the delta component. This should however result in biphasic scatchard plots, since DHM would competitively lower the apparent affinity of DADL to mu, but not delta sites. We therefore interpret these findings in terms of an interaction of DADL and DHM with different, allosterically coupled sites on mu receptor molecules (Pfeiffer and Herz, 1982a,b).

According to Michaelis Menton kinetics, noncompetitive inhibition would indicate an interaction with topographically distinct binding sites. In this regard it is noteworthy that cristallographic studies of carboxypeptidase A binding sites have recently shown that noncompetitively interacting ligands can bind to the same binding site on the enzyme (Rees and Libscomb, 1981). Differences in the detailed binding mechanism and in the rates of steps in a multistep binding reaction may determine the kinetic effects of an inhibitor.

A model of the opiate receptor(s) as a binding area involving regions which may be specifically affected by ions and nucleotides (directly or via further proteins) and which interact differently with the various classes of opiate agonists may therefore be appropriate to explain the different effects of these agents on the binding affinities of mu, delta and kappa agonists to each of the opiate receptors.

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