DIFFERENTIAL REGULATION OF THE μ -, δ -, AND κ -OPIATE RECEPTOR SUBTYPES BY GUANYL NUCLEOTIDES AND METAL IONS¹

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Abstract

The effects of Na⁺, guanyl-5'-yl imidodiphosphate (Gpp(NH)p), and Mn²⁺ on the binding of dihydromorphine, ethylketocyclazocine, D-Ala², D-Leu⁵-enkephalin, and diprenorphine to the opiate receptor were investigated. Three distinct binding sites, μ , δ , and κ sites, were identified with the use of multiple tracer displacement curves. Moreover, this approach was used to determine the effects of Gpp(NH)p and metal ions on each individual binding site. At the μ and δ sites, Na⁺ and Gpp(NH)p each decreased and Mn²⁺ increased agonist binding affinities, with the exception of D-Ala², D-Leu⁵-enkephalin affinity which was not affected by Gpp(NH)p. None of these conditions markedly altered dihydromorphine and D-Ala², D-Leu⁵-enkephalin binding to κ sites, whereas the affinity of ethylketocyclazocine for κ sites was decreased by Gpp(NH)p. Sodium ions lowered the capacity of μ sites and Gpp(NH)p reduced that of δ sites, while both agents increased the capacity of apparent κ sites. These results demonstrate that each of the kinetically distinguishable binding sites is regulated differentially by metal ions and guanyl nucleotides. Simultaneous addition of Na⁺ and Gpp(NH)p greatly reduced the binding affinity of all three agonists at their respective high affinity sites (dihydromorphine at the μ site, D-Ala², D-Leu⁵-enkephalin at the δ site, and ethylketocyclazocine at the κ and μ sites). This result confirms previous observations that agonist binding is characterized by a large affinity reduction in the presence of both Na⁺ and guanyl nucleotides, and it extends this concept to each of the opiate receptor subtypes.

Recently, three types of opiate receptors, the μ , δ , and κ binding sites, have been identified in rodent brain (Chang et al., 1981; Kosterlitz et al., 1981; Pfeiffer and Herz, 1981). The μ site is defined by high affinity for dihydromorphine, while the δ site displays high affinity for D-Ala², D-Leu⁵-enkephalin (Lord et al., 1977; Chang and Cuatrecasas, 1979). The κ site was predicted from pharmacological studies (Martin et al., 1976) and possesses high affinity for the κ agonist ethylketocyclazocine, the putative σ agonist SKF 10,047, and the oripavine alkaloids etorphine and diprenorphine but very low affinity for dihydromorphine and D-Ala², D-Leu⁵-enkephalin (e.g., Pfeiffer and Herz, 1981, 1982). However, highly

specific tracers are currently unavailable except those for the μ binding sites. Two methods have been used to overcome the limitations inherent with tracers of moderate selectivity: the selective blocking method that employs unlabeled μ or δ ligands to suppress tracer binding to these sites (Chang et al., 1981; Kosterlitz et al., 1981) and the simultaneous analysis of multiple displacement curves with various tracers and unlabeled ligands (Pfeiffer and Herz, 1981, 1982). The simultaneous computerized, weighted nonlinear least squares regression program (Munson and Rodbard, 1980) is capable of dissecting binding to several sites and has the advantage that it utilizes binding data over the entire displacement curve. Thus, such an approach with multiple tracers may more readily detect deviations from the law of mass action by comparing the parameter estimates obtained with different tracer combinations. Deviations may include cooperativity of binding (which, however, has yet to be demonstrated for the opiate receptor) and the proposed interconversions among the μ and δ sites (Bowen et al., 1981).

Opiate receptor binding has been characterized by the

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effects of metal ions and guanyl nucleotides on agonist and antagonist receptor interactions (Simantov et al., 1976; Blume, 1978; Childers and Snyder, 1980). Both Na^+ and GTP were shown to be necessary for functional coupling of the opiate receptor to adenylate cyclase in neuroblastoma $\times$ glioma hybrid cells (Blume et al., 1979). These agents potentially decrease agonist binding, while antagonist binding is only slightly decreased or remains unaffected (Blume, 1978; Blume et al., 1979; Childers and Snyder, 1980). Recent results suggest that the μ receptor sites display higher sensitivity toward the effect of GTP than the δ sites (Pert and Taylor, 1979). Similarly, Kostertitz et al. (1981) suggested that the κ sites are less sensitive to Na^+ than the μ sites; however, direct binding data that address the regulation of the κ sites are not available. Furthermore, divalent metal ions (e.g., Mg^{2+} and Mn^{2+}) enhance opiate agonist binding (Pasternak et al., 1975) and affect opiate function, as these ions antagonize opiate effects *in vivo* (Chapman and Way, 1980). The differential effects of the divalent metal ions on the opiate receptor subtypes are largely unknown.

In these experiments, the effects of Na^+ , guanyl-5'-yl imidodiphosphate (Gpp(NH)p), and Mn^{2+} were studied on the binding capacities and affinities of various opiate ligands for the three subclasses of binding sites. Estimates of the binding parameters were obtained by simultaneous computer analysis of multiple displacement curves using the prototypic ligands of the μ , δ , and κ sites (i.e., dihydromorphine, D-Ala², D-Leu⁵-enkephalin, and ethylketocyclazocine) together with the opiate antagonist diprenorphine that labels all sites with similar affinity. The results support the view that each site is regulated differentially by Na^+ , Mn^{2+} , and Gpp(NH)p; however, the large reduction of agonist affinity by the combined action of Na^+ and guanyl nucleotide that is thought to characterize opiate agonist binding did occur at each receptor subtype.

Materials and Methods

Male Sprague-Dawley rats weighing 160 to 280 gm were decapitated and their brains were removed rapidly. The cerebella then were excised and the remainder was placed in 20 vol of ice cold 50 mM Tris-HCl, pH 7.4, and homogenized with an Ultra Turrax homogenizer (twice for 10 sec). This homogenate was either used directly (untreated membranes) or centrifuged at $38,000 \times g$ for 20 min at 4°C. The resulting pellet was resuspended in Tris buffer kept on ice for 30 min and recentrifuged as before (washed membranes). For binding assays, the tissue was diluted to a final concentration of 1:200. This dilution was found to be required in order to obtain a linear dependence of [³H]diprenorphine binding on the tissue concentration. Incubations were performed at 35°C under equilibrium conditions (W. Sadée, A. Pfeiffer, and A. Herz, manuscript in preparation), i.e., for 35 min in untreated membranes and in the presence of Na^+ and Gpp(NH)p, for 60 min in washed membranes, and for 90 min in the presence of Mn^{2+} . For the construction of displacement curves, 10 to 15 concentrations of unlabeled displacer were incubated in 4-ml portions, with a fixed concentration of the labeled ligand ranging between 0.1 and 0.4 nM for [³H]diprenorphine and 0.3 and 1.2 nM for

the other labeled ligands. Specific binding of [³H]diprenorphine (calculated by subtraction of binding in the presence of 1 μM diprenorphine) was 85 to 93%. For the other ligands, specific binding amounted to 70 to 85% in Tris buffer and in the presence of 1 mM Mn^{2+} and ranged between 50 and 70% in the presence of 60 μM Gpp(NH)p and 100 mM Na^+ .

Data analysis. The weighted nonlinear least squares regression program, "Ligand," developed by Munson and Rodbard (1980) was used for quantitative analysis of the experimental data. (For a detailed description, see Munson and Rodbard, 1980.) In brief, the method, based on the mass action law for interaction of multiple ligands with multiple binding sites, provides estimates ($\pm$ SE) for the affinities of the ligands, the nonspecific binding of the ligands, and the capacities of the binding sites. For each displacement curve or combination of displacement curves, fitted estimates are provided for the data assuming one, two, or three binding sites, with the deviation of observed points from predicted values being weighted according to the reciprocal of the predicted variance. Statistical analysis comparing the "goodness of fit" (based on an *F* test incorporated with the program) was used to determine the most appropriate model of binding. In order to test the significance of differences between the parameter estimates under the different conditions, the binding affinities and capacities were constrained to control values (unwashed membranes, Tris buffer alone). This resulted in significantly worse fits ($p < 0.005$) under all different conditions. Since the differentiation of μ , δ , and κ sites required simultaneous computer evaluation of multiple tracer displacement curves (for details, see Pfeiffer and Herz, 1982), estimates of K_d and B_{max} for the individual binding sites (reported in Table I) were based on the overall fits of multiple displacement curves of all four tracers. Similar estimates were obtained under each experimental condition indicated except in the presence of both Na^+ and Gpp(NH)p where only [³H]diprenorphine was used. In order to test whether [³H]diprenorphine displayed different affinities for the three sites distinguished, the effect of forcing K_d values for diprenorphine to a common value on the goodness of fit was evaluated. Since sharing K_d values did not worsen the fit under various conditions significantly, diprenorphine was assumed to display identical affinities for the three distinguished binding sites.

Materials. D-Ala², D-Leu⁵-[³H]enkephalin (42 Ci/mmol), [³H]dihydromorphine (73.2 Ci/mmol), and [³H]diprenorphine (9.1 Ci/mmol) were purchased from Amersham Buchler and [³H]ethylketocyclazocine (15 Ci/mmol) was from New England Nuclear. Unlabeled D-Ala², D-Leu⁵-enkephalin was from Bachem, Bubendorf, Switzerland, dihydromorphine was from Merck, diprenorphine was from Reckitt and Colman, and ethylketocyclazocine was from Sterling Winthrop.

Results

Displacement curves were established for [³H]diprenorphine, [³H]ethylketocyclazocine, D-Ala², D-Leu⁵-[³H]enkephalin, and [³H]dihydromorphine against each of the same four unlabeled ligands. The computer program was used to first establish individual fits of the displacement curves and then simultaneous fits of data obtained

using two or three ligands and finally four ligands together. Parameter estimates obtained by the various combinations were in agreement with those obtained when analyzing the four ligands together. As shown in Figure 1, a typical pattern of displacement curves is obtained when displacing [^{3} H]diprenorphine by unlabeled diprenorphine, ethylketocyclazocine, dihydromorphine, or D-Ala ^{2} , D-Leu ^{5} -enkephalin in rat brain membranes. Ethylketocyclazocine displaces the total binding with a steep slope. In contrast, with dihydromorphine, a shallow curve is apparent, whereas the isotherm with D-Ala ^{2} , D-Leu ^{5} -enkephalin may be described as biphasic with an initial steep slope and a second rather shallow component (Fig. 1). In agreement with previous results (Pfeiffer and Herz, 1981), a three-site model was found to represent the experimental data significantly better than a two-site model, suggesting a μ , a δ , and a third (κ) binding component. A similar analysis was performed using data obtained in untreated rat brain membranes in the presence of either Mn $^{2+}$, Na $^+$, or Gpp(NH)p, a stable analogue of GTP. A three-site binding pattern was apparent with Na $^+$, Gpp(NH)p, and Mn $^{2+}$. However, the binding capacities of the three sites and affinities of the opiate agonists were affected differently (Table I). The binding affinity and capacity of the opiate antagonist diprenorphine was only little affected by the various additions (see legend to Table I).

Na $^+$. One hundred millimolar Na $^+$ strongly decreased the binding affinity of dihydromorphine, and that of D-Ala ^{2} , D-Leu ^{5} -enkephalin and ethylketocyclazocine to μ and to δ sites was somewhat less decreased. This change is reflected by a shift to the right of the displacement curves against the antagonist (Figs. 2, 3, and 4). The

binding affinities of the agonists at κ sites were unaffected. The binding capacity of δ sites was reduced by 50% in the presence of Na $^+$. In contrast, the capacity of κ sites was doubled and that of μ sites was unchanged.

Gpp(NH)p. Sixty micromolar Gpp(NH)p decreased the affinity of dihydromorphine at μ and δ sites 2-fold. D-Ala ^{2} , D-Leu ^{5} -enkephalin had a decreased affinity at δ sites, whereas no change was observed at μ and κ sites. The affinity of ethylketocyclazocine was similarly reduced at all three sites. These changes in affinities were reflected in moderate shifts to the right of the displacement curves (Figs. 2, 3, and 4). The binding capacity of μ sites was decreased by 50%. In contrast, κ sites showed a marked increase in number, whereas δ sites were little affected by Gpp(NH)p. These changes in capacities also were reflected in the displacement curves, which show a considerably more important shallow part of the displacement isotherm of D-Ala ^{2} , D-Leu ^{5} -enkephalin against [^{3} H]diprenorphine.

Gpp(NH)p and Na $^+$. The combined presence of Gpp(NH)p and Na $^+$ almost completely abolished the specific binding of [^{3} H]dihydromorphine and D-Ala ^{2} , D-Leu ^{5} -[^{3} H]enkephalin and greatly decreased that of [^{3} H]ethylketocyclazocine. To delineate binding affinities and capacities, displacement experiments using only [^{3} H]diprenorphine as the radioligand were performed. The data, presented in Figures 2, 3, and 4 and Table II, show that displacement curves of the agonists were shifted to the right relative to curves obtained in the presence of either Gpp(NH)p or Na $^+$ alone. The displacement curve of D-Ala ^{2} , D-Leu ^{5} -enkephalin was shallow overall and had lost the initial steeper slope. Computer modeling permitted the distinction of two binding sites with D-Ala ^{2} , D-Leu ^{5} -enkephalin and dihydromorphine, a smaller component with higher affinity and a larger component with lower affinity. Ethylketocyclazocine displaced [^{3} H]diprenorphine with intermediate affinity.

Mn $^{2+}$. In the presence of 1 mM Mn $^{2+}$, opiate agonists had an enhanced binding affinity at μ sites and, to a lesser extent, at δ sites, whereas little changes in binding affinities occurred at κ sites. Mn $^{2+}$ strongly enhanced the binding capacity of the δ site while leaving that of the other sites unchanged. The displacement curves of dihydromorphine and, more pronounced, that of D-Ala ^{2} , D-Leu ^{5} -enkephalin were shifted to the left, and the steeper part of the curves accounted for the major component.

Washed membranes. Washing the membranes in ice cold Tris buffer resulted in a series of changes, most notably a 2-fold enhancement of the number of apparent δ sites (Table I). The binding affinities of all opiate agonists were increased at μ sites but not at δ and κ sites. However, there are no qualitative changes of the three-site binding model.

Figure 1. Displacement curve of [^{3} H]diprenorphine against unlabeled diprenorphine, ethylketocyclazocine, dihydromorphine, and D-Ala ^{2} , D-Leu ^{5} -enkephalin in washed brain membranes. The fraction of bound ligand after subtraction of non-specific binding is plotted on the ordinate. The abscissa indicates the total ligand concentration. The lines were obtained in representative experiments from the computer program.

Discussion

In the present study, the effects of metal ions and of the stable GTP analogue Gpp(NH)p on the binding affinities and capacities of opiate prototype agonists at μ , δ , and κ receptor sites were investigated. The experiments were performed in unwashed rat brain membrane homogenates. This design involves minimal homogenate manipulation and was chosen because various *in vitro*

TABLE I

Parameter estimates of the binding capacity and K_d for opiate agonists to μ , δ , and κ binding sites in the absence and presence of 1 mM $MnCl_2$, 60 μM Gpp(NH)p, or 100 mM NaCl and in washed membrane homogenates (Tris buffer alone)

Binding parameters were derived by computer modeling of curves obtained by displacement of labeled dihydromorphine, D-Ala², D-Leu⁵-enkephalin, ethylketocyclazocine, and diprenorphine by each of the unlabeled ligands (i.e., 16 different combinations of labeled ligand and unlabeled displacer were analyzed together). Two displacement curves of each combination (with 12 data points per curve) were obtained in independent experiments. The SE values shown thus reflect inter- and intra-assay variance. The K_d values of diprenorphine were assumed to be identical for the three binding sites and were 0.29 ± 0.05 nM under control conditions (untreated membranes), 0.29 ± 0.05 nM with $MnCl_2$, 0.36 ± 0.05 nM with Gpp(NH)p, 0.33 ± 0.04 nM with NaCl, and 0.31 ± 0.06 nM in washed membrane homogenates. The degrees of freedom varied between 110 and 125 for the experiments. The three-site models were fitted significantly better ($p < 0.01$) than two-site models in all experiments.

Addition	Binding Capacity	K_d		
		Dihydromorphine	D-Ala ² , D-Leu ⁵ -enkephalin	Ethylketocyclazocine
	<i>pmol/gm tissue</i>		<i>nM</i>	
μ receptor				
Control	10.7 $\pm$ 1.1	2.8 $\pm$ 0.4	21.4 $\pm$ 3.1	2.5 $\pm$ 0.3
Mn ²⁺	10.3 $\pm$ 1.9	1.8 $\pm$ 0.2	10.6 $\pm$ 1.8	1.9 $\pm$ 0.3
Gpp(NH)p	5.4 $\pm$ 0.7	4.9 $\pm$ 1.3	17.0 $\pm$ 4.8	4.8 $\pm$ 1.0
NaCl	12.5 $\pm$ 1.2	20.8 $\pm$ 6.8	41.8 $\pm$ 9.7	4.7 $\pm$ 1.2
Washed membranes	9.5 $\pm$ 1.4	2.1 $\pm$ 0.3	10.0 $\pm$ 1.9	1.4 $\pm$ 0.3
δ receptor				
Control	2.2 $\pm$ 0.8	108 $\pm$ 21	1.6 $\pm$ 0.3	10.2 $\pm$ 1.8
Mn ²⁺	4.4 $\pm$ 0.9	73 $\pm$ 14	1.3 $\pm$ 0.4	5.8 $\pm$ 1.4
Gpp(NH)p	2.4 $\pm$ 0.7	233 $\pm$ 112	2.5 $\pm$ 1.0	24.6 $\pm$ 5.1
NaCl	1.2 $\pm$ 0.6	1859 $\pm$ 789	2.9 $\pm$ 1.3	37.2 $\pm$ 13
Washed membranes	4.5 $\pm$ 1.5	152 $\pm$ 26	1.4 $\pm$ 0.3	9.0 $\pm$ 1.5
κ receptor				
Control	2.6 $\pm$ 0.5	385 $\pm$ 126	3160 $\pm$ 928	1.6 $\pm$ 0.4
Mn ²⁺	2.1 $\pm$ 0.5	400 $\pm$ 143	5560 $\pm$ 3740	2.3 $\pm$ 0.8
Gpp(NH)p	3.6 $\pm$ 0.6	313 $\pm$ 104	3780 $\pm$ 1530	6.7 $\pm$ 1.5
NaCl	5.0 $\pm$ 0.7	442 $\pm$ 102	3033 $\pm$ 819	3.1 $\pm$ 1.3
Washed membranes	2.1 $\pm$ 0.5	344 $\pm$ 175	5570 $\pm$ 3630	0.7 $\pm$ 0.3

Figure 2. Displacement of [³H]diprenorphine by D-Ala², D-Leu⁵-enkephalin in unwashed membranes in the presence and absence of 1 mM Mn^{2+} , 100 mM Na^+ , 60 μM Gpp(NH)p, and 60 μM Gpp(NH)p plus 100 mM Na^+ .

Figure 3. Displacement of [³H]diprenorphine by unlabeled dihydromorphine in untreated membranes in the absence and presence of Mn^{2+} , 100 mM Na^+ , 60 μM Gpp(NH)p, and 60 μM Gpp(NH)p plus 100 mM Na^+ .

membrane treatments were shown to affect opiate binding (Simantov et al., 1976; W. Sadée, A. Pfeiffer, and A. Herz, manuscript in preparation). Comparison of the binding in untreated and washed rat brain membrane homogenates showed some changes in the binding affin-

ities and capacities (Table I), but the binding kinetics remained qualitatively similar after membrane washing.

Computerized analysis of multiple displacement curves with various unlabeled ligands can be performed with either one tracer alone, with a combination of two or

Figure 4. Displacement of [^3H]diprenorphine by unlabeled ethylketocyclazocine in untreated membranes in the absence and presence of 1 mM Mn^{2+} , 100 mM Na^+ , 60 μM Gpp(NH)p, and 60 μM Gpp(NH)p plus 100 mM Na^+ .

TABLE II

Parameter estimates of the binding capacity and K_d for dihydromorphine, D-Ala ^{2} , D-Leu ^{5} -enkephalin, and ethylketocyclazocine from computer modeling of displacement curves against [^3H]diprenorphine in the presence of 60 μM Gpp(NH)p plus 100 mM NaCl

Parameter estimates were obtained by fitting three or four displacement curves for each agonist which were replicated in independent experiments. R_1 and R_2 represent distinct binding sites.

Displacer	Binding Capacity		K_d	
	R_1	R_2	R_1	R_2
	pmol/gm		nM	
D-Ala ^{2} , D-Leu ^{5} -enkephalin	7.2 $\pm$ 1.3	10.2 $\pm$ 1.7	32.8 $\pm$ 19	3290 $\pm$ 509
Ethylketocyclazocine	17.1 $\pm$ 1.6		30.2 $\pm$ 1.8	
Dihydromorphine	5.9 $\pm$ 1.5	12.0 $\pm$ 1.9	26.5 $\pm$ 16	1120 $\pm$ 205

three tracers, or with all of the four tracers used in this study. Previous experiments performed with three different tracers demonstrated that, in each case, a three-site model gave the best fit of the displacement curves (Pfeiffer and Herz, 1982). Moreover, similar values were obtained for receptor capacities and affinities regardless of the tracer combination, and the data were not altered with the simultaneous use of four tracers. This result supports the hypothesis that opiate binding occurred at three independent sites that obey the law of mass action under the experimental conditions. When homogenates were incubated in the presence of both NaCl and Gpp(NH)p, agonist binding was too low for accurate analysis of the displacement curves. However, binding of the antagonist tracer [^3H]diprenorphine was not reduced significantly in affinity or capacity. Multiple [^3H]diprenorphine displacement curves were compatible with a two-site model (Table II), but analysis of the nature and multiplicity of these sites was limited.

The affinities of the opiate agonists were generally changed in similar directions at μ and δ sites under the various experimental conditions. Thus, NaCl and Gpp(NH)p decreased, and MnCl_2 increased the agonist affinities. Although qualitatively similar, binding of the μ agonist dihydromorphine was more strongly affected than that of D-Ala ^{2} , D-Leu ^{5} -enkephalin and ethylketocyclazocine. Neither agent seemed to affect binding of dihydromorphine and D-Ala ^{2} , D-Leu ^{5} -enkephalin to κ sites to any significant degree, although the rather large error estimates preclude the detection of small changes that might have occurred. In contrast, the affinity of ethylketocyclazocine at κ sites was decreased 4-fold by Gpp(NH)p. The δ binding capacity of D-Ala ^{2} , D-Leu ^{5} -enkephalin was increased markedly by MnCl_2 and decreased by NaCl, whereas the capacity of μ sites was decreased selectively by Gpp(NH)p. The apparent κ binding capacity was increased by NaCl and Gpp(NH)p and, thus, was modulated in the opposite direction to μ sites with Gpp(NH)p and δ sites with NaCl.

With regard to μ and δ sites, these results are in agreement with previous publications. Zukin et al. (1980) also reported a loss of D-Ala ^{2} , D-Leu ^{5} -enkephalin affinity without apparent change in the number of binding sites by GTP. Blume et al. (1979) have shown that NaCl apparently decreased the agonist binding capacity of δ sites in neuroblastoma $\times$ glioma hybrid cells, which was actually the result of a large decrease in agonist binding affinity to about half of the sites that were detectable by antagonist tracer binding. Furthermore, a decrease in the binding capacity of dihydromorphine by GTP has been reported by Blume (1978) and by Childers and Snyder (1980). These authors also found a 3-fold and 10- to 20-fold decrease in dihydromorphine and D-Ala ^{2} , D-Leu ^{5} -enkephalin affinity by GTP and NaCl, respectively. Chang and Cuatrecasas (1979) had shown an increase in D-Ala ^{2} , D-Leu ^{5} -enkephalin binding capacity by MnCl_2 . Such an increase also has been reported for dihydromorphine in the presence of MnCl_2 , which was, however, dependent upon the incubation conditions (Simantov et al., 1976).

The combined presence of NaCl and Gpp(NH)p further strongly decreased the binding affinities of all three agonists. A large affinity reduction by Na^+ plus GTP was proposed to characterize opiate agonist binding (Blume, 1978; Childers and Snyder, 1980). The data presented here demonstrate that this effect occurs at all receptor subsites, i.e., for dihydromorphine at the μ site, for D-Ala ^{2} , D-Leu ^{5} -enkephalin at the δ site, and for ethylketocyclazocine at the κ and μ sites. Therefore, ethylketocyclazocine may act as an agonist at both the μ and κ binding sites. Moreover, the diprenorphine displacement curves show that the overall binding capacity (~ 17 pmol/gm of tissue; Table II) was not changed by NaCl and Gpp(NH)p and that all agonists retain the ability to displace [^3H]diprenorphine, although at a much reduced affinity. Therefore, it is conceivable that NaCl and Gpp(NH)p alter the receptor such that the binding of agonists but not antagonists induces a major change in receptor configuration (e.g., coupling to an effector).

In summary, there are three major results. (i) Agonist binding affinities were affected differentially by Na^+ , Mn^{2+} , and Gpp(NH)p at the μ , δ , and κ binding sites.

This probably reflects differences in the structure and composition of the three classes of binding sites. (ii) The binding capacity of apparent κ sites seemed to be regulated in the opposite direction to μ and δ sites by Na^+ and Gpp(NH)p. However, the decrease in δ and μ sites was not equivalent to the increase in κ sites, which argues against interconversion of μ or δ sites into κ sites. (iii) All three types of opiate agonists display large decreases in binding affinities to their respective high affinity sites in the presence of Na^+ plus Gpp(NH)p. This finding suggests that all three opiate receptor subclasses, μ , δ , and κ , are similarly coupled via a GTP regulatory unit, possibly to adenylate cyclase.

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