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Sodium Regulation of Agonist Binding at Opioid Receptors. II. Effects of Sodium Replacement on Opioid Binding in Guinea Pig Cortical Membranes

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SUMMARY

We have examined the effects of sodium on the binding of opioid agonists to μ-, δ-, and κ-receptors in guinea pig cortical membranes. Concentration curves for sodium indicated that maximal inhibition of μ binding by this cation was about 60% and maximal inhibition for δ binding was about 70%, whereas that for κ binding was only about 20%. The concentration of sodium required for half-maximal inhibition of binding to all three sites was about 10–30 mM, corresponding to the intracellular sodium concentration. The nature of the sodium effect was further characterized by saturation analysis of binding to each of the three receptor types by comparing results obtained in the presence of 120 mM sodium with those obtained with equimolar replacement of sodium by another cation. Two radiolabeled agonists with different structural characteristics were tested for each binding site. In the presence of sodium, the affinity of the labeled agonists for μ sites was approximately 2–3-fold less than in its absence, but the density of binding sites was not changed. At κ sites, sodium reduced agonist affinity slightly but, again, did not alter the number of binding sites. In contrast, sodium reduced the apparent density of δ-binding sites while leaving the agonist affinity unchanged. Competition against antagonist binding to δ sites indicated that, in the presence of sodium, a higher proportion of sites was in a lower affinity state, as reflected by the biphasic nature of the agonist displacement curve. In contrast, the effect of sodium on displacement of antagonist from μ sites was to lower the affinity of the agonist. Competition against antagonist binding to κ sites also showed a reduction in agonist affinity by sodium, but no change in numbers of receptors. The results indicate that sodium may differentially regulate agonist binding to opioid receptor types and that this regulation may occur at an intracellular site. The κ site appears to be less sensitive to sodium than the μ and δ sites.

Sodium variably regulates the binding of opioids to their receptors. Although this effect has generally been regarded as due to a reduction in agonist affinity brought about by the presence of sodium ions (1, 2), others have suggested that the lower amount of binding is due to a decrease in the apparent B_{max} (density) of the receptors (3–5). We have sought to resolve this discrepancy by investigating the effect of sodium on each of the three best characterized opioid-binding sites, μ, δ, and κ. We have shown in the preceding paper (6) that sodium produces a 2–3-fold reduction of agonist binding affinity to receptors with μ-like properties in 7315c pituitary tumor cells. In contrast, sodium appears to produce a large reduction in affinity of agonist binding to about one half of the δ-binding sites in NG108-15 neuroblastoma × glioma hybrid cells, but it has no effect on agonist binding to the remaining binding sites.

We have previously reported (7) that, in guinea pig cortical membranes, [^{3}H]DAGO binds to a single population of sites with μ-type characteristics, [^{3}H]DADLE in the presence of 10 nM DAGO binds to a single population of δ sites, and [^{3}H]EKC in the presence of 1 μM DAGO binds to a single population of κ sites. In this paper we examine the effects of varying sodium concentrations on the binding of these and other agonists to μ-, δ-, and κ-receptors at constant ionic strength, maintained by equimolar replacement of sodium by other cations. A preliminary report of some of these studies has been published (8).

Materials and Methods

Reagents. [^{3}H]DAGO (60 Ci/mmol), [^{3}H]DADLE (36 Ci/mmol), [^{3}H]etorphine (46 Ci/mmol), [^{3}H]DHM (65 Ci/mmol), and [^{3}H]DIP

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the view of the Department of Defense or the Uniformed Services University of the Health Sciences. The experiments reported herein were conducted according to the principles set forth in the *Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. [NIH] 78-23, 1978).

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ABBREVIATIONS: DAGO, Tyr-D-Ala-Gly-(Me)Phe-Gly-ol; DADLE, [D-Ala^2-D-Leu^5]enkephalin; DSLET, [D-Ser^2,Leu^5]enkephalin-Thr; DPDPE, [D-Pen^2-D-Pen^5]enkephalin; U50,488H, *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide methanesulfonate hydrate; EKC, ethylketocyclazocine; DIP, diprenorphine; ICI 174,864, allyl$_2$-Tyr-(α-aminoisobutyric acid)$_2$-Phe-Leu-OH; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; N-MG, *N*-methyl-D-glucamine, K_D, dissociation constant of labeled ligand; K_i, dissociation constant of displacing ligand.

were purchased from Amersham (Arlington Heights, IL). [^{3}H]EKC (24 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Unlabeled DAGO, DADLE, and ICI174,864 were obtained from Cambridge Research Biochemicals, Ltd. (Atlantic Beach, NY). DSLET was purchased from Sigma Chemical Co. (St. Louis, MO). DPDPE was purchased from VEGA (Tucson, AZ). EKC methane sulfonate and levallorphan were gifts of Sterling Winthrop (Rensselaer, NY) and U50,488H was a gift from The Upjohn Company (Kalamazoo, MI).

Preparation of neural membranes. Male Hartley guinea pigs (250–500 g; Controlled Animal Management and Marketing, Wayne, NJ) were sacrificed by decapitation and their brains were removed to ice. Cortices were dissected and homogenized in 10 volumes modified Krebs-HEPES buffer (NaCl, 118 mM; KCl, 4.8 mM; $CaCl_2$, 2.5 mM; $MgCl_2$, 1.2 mM; HEPES, 25 mM; pH adjusted to 7.4) with a Teflon glass homogenizer driven by a T-line laboratory stirrer (Thomas Scientific, Philadelphia, PA) at a setting of 70. The homogenate was centrifuged at 27,000 × *g* for 15 min at 4°. The pellet was resuspended in 20 volumes of buffer and held on ice for 60 min to remove any endogenous ligand. The suspension was then washed three times by centrifugation at 27,000 × *g* for 15 min at 4° and resuspended in ice-cold buffer. Membranes were stored frozen in modified Krebs-HEPES buffer at −70° at a concentration of 2% (w/v).

Binding assay. Membranes were thawed at room temperature and homogenized by five strokes in a Teflon glass homogenizer. For binding assays in which sodium was replaced by potassium or N-MG, the membranes were washed twice in buffer containing an equimolar concentration of the appropriate cation. The wash procedure consisted of centrifuging the membrane suspension at 27,000 × *g* for 15 min at 4° and resuspending in the replacement buffer. Membranes used in parallel experiments were washed in sodium containing buffer. After all additions, the final membrane concentration in the assay tube was 1%, corresponding to about 300–500 μg of protein/sample. All drug solutions were prepared in Krebs-HEPES buffer, pH 7.4, with or without replacement cations as appropriate. The final volume in each tube was 500 μl. Triplicate samples of membrane suspension were preincubated for 5 min with or without nonradioactive displacer. Radiolabeled ligand was then added and the incubation continued for 20 min, by which time all labeled ligands used achieved equilibrium. The incubation was terminated by the addition of 4 ml of ice-cold buffer and rapid filtration through Schleicher and Schuell No. 32 or Whatman GF/B glass fiber filters under reduced pressure. The filters were washed with an additional 8 ml of buffer and transferred to scintillation vials. One ml of absolute ethanol and 8 ml of Beckman EP Ready-Solv were added to samples, which were then counted at an efficiency of about 40%. For saturation experiments, binding was measured at 12–18 concentrations between 0.05 and 50 nM. In order to achieve high concentrations of some ligands, nonradioactive ligand was added to radioactive, and the specific activity was adjusted accordingly in the data reduction. For displacement studies, 15–18 concentrations of nonradioactive displacer were used against a single concentration of labeled agonist, chosen to be below the K_D of that ligand at the preferred site, except in the case of [^{3}H]DADLE, used at a concentration of 5 nM, and [^{3}H]etorphine, used at 2 nM, in order to obtain sufficient counts to construct displacement curves. For competition against antagonist, 24 concentrations of displacer were used. [^{3}H]DAGO or [^{3}H]DHM was used to characterize binding to μ sites, [^{3}H]DADLE in the presence of 10 nM DAGO or [^{3}H]etorphine in the presence of 1 μM DAGO and 100 nM U50,488H was used to characterize binding to δ sites, and [^{3}H]EKC in the presence of 1 μM DAGO or [^{3}H]etorphine in the presence of 1 μM DAGO and 100 nM DSLET was used to characterize binding to κ sites. For studies of competition against antagonist binding to μ sites, 1 nM [^{3}H]DIP was bound in the presence of 1 μM ICI 174,864 and 500 nM U50,488H. The same concentration of [^{3}H]DIP was bound for competition against δ antagonist binding in the presence of 1 μM DAGO and 1 μM U50,488H. For studies of competition against antagonist binding to κ sites, 1 nM [^{3}H]DIP was bound in the presence of 1 μM DAGO and 500 nM ICI 174,864. These concentrations of site-selective displacing ligands were chosen from analysis of their potencies as displacers of each labeled ligand. Nonspecific binding was defined as the fraction of bound radioligand that remained in the presence of 1 μM unlabeled DAGO (μ), 100 nM or 1 μM DSLET (δ), and 1 μM EKC or 1 μM U50,488H (κ). Specific binding to membranes was always less than 4% of added radioligand.

Analysis of binding data. Saturation and displacement data were analyzed by the use of the computer program LIGAND (9). This program utilizes a nonlinear least squares curve-fitting algorithm and assumes the simultaneous contribution of one or more binding sites. The K_D values reported herein are the dissociation constants derived by the program for the labeled ligands, and the K_i values are the dissociation constants derived for the unlabeled displacers. All possible models in which like parameters from "control" and "treated" membranes were either constrained to be equal or allowed to float were fitted to the data. In each case, the model reported fit significantly better than all others according to the F test at $p < 0.001$ unless otherwise stated.

Stability of labeled and unlabeled compounds. The stability of ligands used was previously confirmed as reported in Werling et al. (7).

Results

In order to determine how sodium was affecting binding to each type of opioid receptor, sodium concentration curves were constructed for the binding of [^{3}H]DAGO to μ sites, [^{3}H]DADLE to δ sites, and [^{3}H]EKC to κ sites. Equimolar potassium replaced sodium in samples where the concentration of sodium was reduced below 120 mM. Results of experiments in which binding to μ, δ, and κ sites was measured at increasing sodium concentrations are shown in Fig. 1. Maximal inhibition by sodium observed at μ sites was about 60%, whereas that for δ binding was about 70%. At κ sites, the greatest inhibition achieved by sodium was only about 20%. For μ, δ, and κ sites, the concentration of sodium giving half-maximal inhibition was about 10–30 mM.

The nature of the sodium-induced inhibition of agonist binding was further examined by saturation analysis of binding at each site in the presence and absence of sodium. In additional experiments, we have now confirmed our preliminary observations (8) that sodium produces a 2–3-fold reduction in [^{3}H]DAGO affinity at μ-binding sites without changing the number of sites (Table 1). In order to confirm that this effect was not specific to [^{3}H]DAGO, binding of [^{3}H]DHM was also examined. In the presence of sodium, this agonist had a K_D of about 9 nM. Binding was displaced by unlabeled DHM and DAGO with K_i values of 8.6 and 5.0 nM. DSLET displaced [^{3}H]DHM with a K_i of about 100 nM, consistent with DSLET affinity for μ sites (7). In saturation analysis of [^{3}H]DHM binding, the inclusion of 120 mM sodium reduced the affinity of [^{3}H]DHM to about one half the value measured in the presence of an equimolar concentration of potassium, but did not affect the number of binding sites (Table 1).

[^{3}H]DADLE, under conditions which allow its binding only to δ sites (7) bound with the same affinity in the presence of 120 mM sodium or an equimolar concentration of potassium. However, the apparent B_{max} was about twice as great in the absence of sodium as in its presence. The alternate ligand used for δ binding was [^{3}H]etorphine in the presence of concentrations of DAGO and U50,488H which block μ and κ sites, respectively. Nonspecific binding was defined as that which remained in the presence of 100 nM DSLET. Residual binding could then be completely displaced by the selective δ agonist,

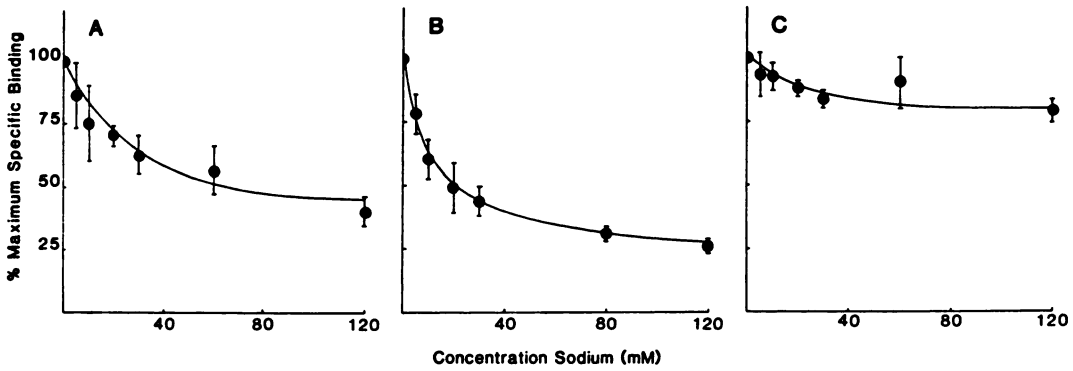

Fig. 1. Effects of increasing concentrations of sodium on binding to μ- (A), δ- (B), and κ- (C) receptors. [^{3}H]DAGO was used to characterize binding to μ sites, [^{3}H]DADLE in the presence of 10 nM DAGO was used to characterize binding to δ sites, and [^{3}H]EKC in the presence of 1 μM DAGO was used to characterize binding to κ sites. Where sodium was reduced to a concentration below 120 mM, an equimolar ionic concentration was achieved by replacement with potassium. Other details of the incubation are given in Materials and Methods. We have previously shown that the receptor selectivities of these ligands in sodium-containing buffer are similar to their selectivities in sodium-free buffer (7).

TABLE 1

Parameters for binding of radioligands to guinea pig cortical membranes: effects of sodium

Parameters were derived by the computer program LIGAND, which uses a nonlinear least squares curve-fitting algorithm to analyze saturation data (8). K_D values are given as nM ± SE and B_{max} values are fmol/mg of protein ± SE. Two or three full saturation analyses were conducted independently for each set of K_D and B_{max} values reported. For experiments in which the binding assays were conducted in sodium-free buffer, this cation was replaced by an equimolar concentration of potassium. In each case, the data were better fit by a single-site model than by more complex models. Other details of the incubation are provided in Materials and Methods.

Site	Radioligand	+Na		−Na	
		K_D	B_{max}	K_D	B_{max}
μ	DAGO	6.7 ± 1.1	33 ± 3.9	2.4 ± 0.61	41 ± 3.9
	DHM	8.6 ± 2.3	52 ± 9.2	4.8 ± 0.54	54 ± 3.8
δ	DADLE	3.5 ± 0.35	20 ± 1.4	2.9 ± 0.22	55 ± 2.6
	ETOR	2.6 ± 0.63	28 ± 4.7	2.7 ± 0.28	60 ± 5.7
κ	EKC	1.2 ± 0.51	110 ± 21	0.7 ± 0.14	113 ± 14
	ETOR	0.97 ± 0.05	89 ± 3.5	0.50 ± 0.06	96 ± 9.9

DPDPE (10, 11), with a K_i of 1.9 nM, supporting the conclusion that, under these conditions, the specific binding of etorphine was to δ-type sites. The data were better fit to a one-site than to a more complex model as determined by the LIGAND program. In saturation experiments, the affinities of the ligand for δ sites remained unchanged (Fig. 2B). As also found for [^{3}H]DADLE, the apparent B_{max} for the binding of [^{3}H]etorphine to δ sites was about twice as great in the absence of 120 mM sodium as in its presence (Table 1).

The K_D of [^{3}H]EKC for κ sites was increased by the presence of sodium from 0.7 to 1.2 nM (Table 1). This sodium-induced affinity change was significant according to the F test at a $p <$ 0.05 level. [^{3}H]Etorphine was also used as the alternate ligand for κ sites. In this situation, DAGO was used to block binding to μ sites, and DSLET to block binding to δ sites. Nonspecific binding was defined as that remaining in the presence of 1 μM U50,488H. Under these conditions, specific binding was fully displaceable by EKC with a K_i of about 1.0 nM, confirming that only κ sites were being labeled by the [^{3}H]etorphine. The data were best fit to a one-site model. The affinity of [^{3}H]etorphine for κ sites was also reduced by sodium (Fig. 2C), reflected in an increase in the K_D from 0.50 to 0.97 (Table 1). There was no change in the B_{max} for either radiolabeled ligand used to identify κ sites.

In order to determine whether the elevated potassium concentration in the sodium-free medium might have itself affected the specific binding, saturation analysis in which sodium was replaced by an equimolar concentration of N-MG was performed for each of the three receptor types (Fig. 3). [^{3}H]DHM binding to μ-receptors in the presence of 120 mM sodium yielded a K_D of about 10 ± 3.3 nM, but when sodium was replaced with N-MG, the K_D was decreased to 3.1 ± 1.5 nM. Under both conditions, the density of receptors was 45 ± 4.1 fmol/mg of protein. [^{3}H]DADLE binding to δ-receptors in the presence of sodium or N-MG yielded a K_D of 3.9 ± 1.1 nM. The B_{max} in the presence of sodium was 34 ± 7.8 fmol/mg of protein whereas, in its absence, the B_{max} was about 62 ± 11 fmol/mg of protein. [^{3}H]EKC binding to κ-receptors again showed a modest increase in affinity when no sodium was included in the incubation medium, reflected by a decrease in the K_D from 1.5 ± 0.36 nM to 0.86 ± 0.11 nM, whereas the density of receptors was 130 ± 13 fmol/mg of protein in either case. These results indicated that the changes observed in the potassium replacement studies were in fact attributable to the absence of sodium and not to the presence of potassium.

In order to further analyze the effects of sodium at the δ site, we performed saturation and displacement experiments using the antagonist [^{3}H]DIP under conditions allowing its binding to δ sites only. Computer analysis of these data indicated that the affinity of [^{3}H]DIP in the presence of sodium was 1.5 nM and in the absence of sodium was 1.8 nM. In the absence of sodium, a biphasic curve was produced by displacement with the δ-preferring ligand DSLET (Fig. 4). The first phase of the displacement yielded a K_i for DSLET of 9 nM, consistent with its K_i as determined from displacement of agonist (7, 10). A second phase of displacement occurred over the range of 50 nM–10 μM. The K_i for DSLET at this site was about 500 nM. In the absence of sodium, the ratio of high to low affinity sites was 2:1. When sodium was replaced by an equimolar concentration of potassium, the K_D of DSLET for the higher and lower affinity sites remained unchanged, but the proportion of high to low sites was now 1:2. The total number of δ sites bound by antagonist remained the same.

The K_D for [^{3}H]DIP at μ sites was 0.1 nM in the presence or absence of sodium. However, in contrast to the results obtained at the δ site, displacement of [^{3}H]DIP binding to μ sites by DAGO in the presence of sodium showed a rightward shift relative to displacement in its absence, but in neither case was

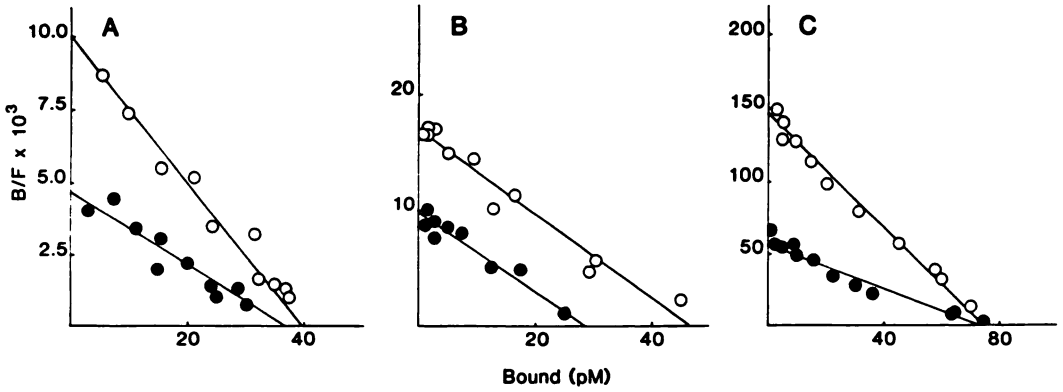

Fig. 2. Scatchard transformation of saturation data for binding to μ (A), δ (B), and κ (C) sites in guinea pig cortical membranes in the presence (●) or absence (○) of 120 mM sodium. Binding to μ sites was characterized by [^{3}H]DHM; binding to δ and κ sites was characterized by [^{3}H]etorphine with appropriate blockers present as described in Materials and Methods. Under these conditions, the data could be best fit by a one-site model in each case. In the absence of sodium, isoosmotic strength was maintained by replacement with potassium. Data are from single experiments that were replicated with similar results. B_{max} values after protein correction were: (μ) sodium, 47 fmol/mg of protein, no sodium, 50 fmol/mg of protein; (δ) sodium, 30 fmol/mg of protein, no sodium, 60 fmol/mg of protein; (κ) sodium, 69 fmol/mg and no sodium, 75 fmol/mg of protein.

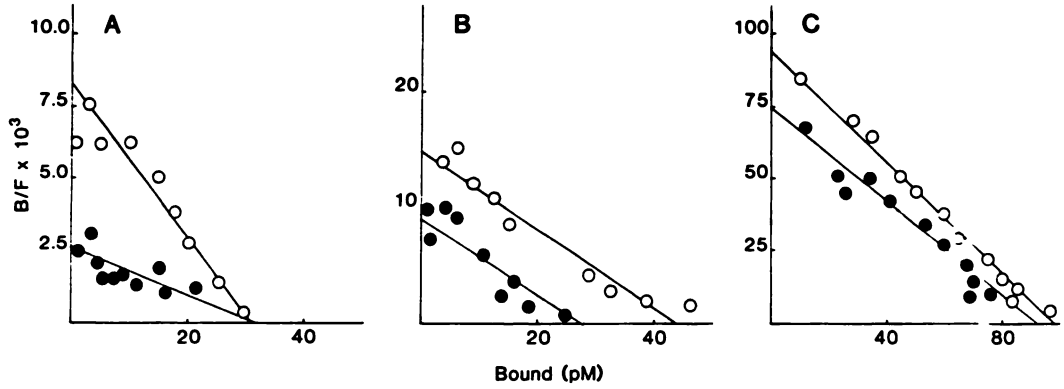

Fig. 3. Scatchard transformation of saturation data for binding to μ- (A), δ- (B), and κ- (C) receptors in the presence (●) and absence (○) of sodium. Binding to μ sites was characterized by [^{3}H]DHM, binding to δ sites was characterized by [^{3}H]DADLE in the presence of 10 nM DAGO, and binding to κ sites was characterized by [^{3}H]EKC in the presence of 1 μM DAGO. Other details are provided in Materials and Methods. Under these conditions, data could be best fit to a single-site model in each case. In the absence of sodium, an isoosmotic strength was achieved by replacement with N-MG. Data are from single experiments. B_{max} values after protein correction were: (μ) sodium, 45 fmol/mg of protein, no sodium, 45 fmol/mg of protein; (δ) sodium, 34 fmol/mg of protein, no sodium, 62 fmol/mg of protein; (κ) sodium and no sodium, 128 fmol/mg of protein.

there evidence for heterogeneity of binding sites (Fig. 5). DAGO displaced [^{3}H]DIP with a K_i of 2.4 nM in the absence of sodium and 6.8 nM in its presence. The number of binding sites was the same in either case.

Again, at κ sites, sodium did not affect the K_D of the labeled antagonist, which was 0.8 nM in its presence or absence. When the antagonist [^{3}H]DIP was bound to κ sites, the displacement of binding by U50,488H was shifted rightward slightly by the inclusion of 120 mM sodium, with the K_i in the absence of sodium at 3.9 nM and in its presence at 5.7 nM (Fig. 6). Again, the number of binding sites remained unaffected.

Discussion

The results obtained from this study indicate that the regulation of agonist binding to opioid receptors in guinea pig cortical membranes varies according to receptor type. Also, whereas both μ and δ agonist binding are depressed greatly by low concentrations of sodium, κ agonist binding is relatively less affected by this cation. It is notable that the concentration of sodium giving half-maximal inhibition of binding at both μ and δ sites is in the range of 10–30 nM, corresponding to the intracellular sodium level. This may indicate that sodium regulation of agonist binding to μ and δ sites occurs at an intracellular site. This possibility is more extensively discussed in the preceding paper (6), where data from experiments using the sodium ionophore monensin reflect changes in the binding parameters of NG108-15 and 7315c cells due to alterations in their intracellular sodium levels.

[^{3}H]DHM as well as [^{3}H]DAGO bound to a single population of sites with μ-like properties. The inclusion of 120 mM sodium in the incubation medium decreased the affinity of either ligand 2–3-fold but did not alter the B_{max} value for μ-receptors. Similar changes were found to occur in the μ-type receptors of 7315c cells and membranes (6). When antagonist was bound to the μ-receptor in guinea pig cortical membranes, DAGO displaced with an affinity about 2-fold higher in the absence of sodium than in its presence. The density of binding sites did not change.

[^{3}H]DADLE and [^{3}H]etorphine, under conditions which allowed their binding to δ sites only, bound only half as many sites in the presence of 120 mM sodium as in its absence. The most likely explanation for this observation is that sodium reduces the affinity of about half the δ high affinity sites to a value not measurable by our radiolabeled agonist ligands. In support of this explanation are the results from the agonist

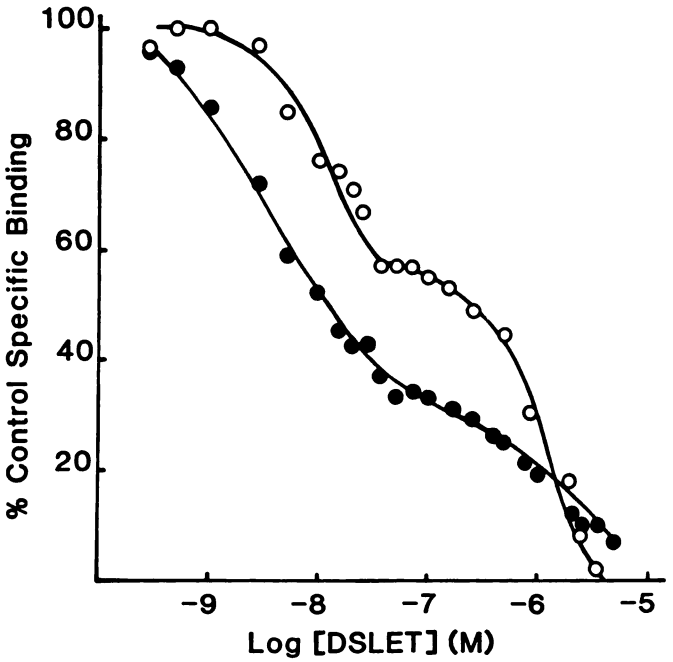

Fig. 4. Competition curve for DSLET against 1 nM [^{3}H]DIP binding to δ sites, with appropriate blockers present as described in Materials and Methods, ○, in the presence of 120 mM sodium, ●, in the absence of sodium (sodium replaced by an equimolar concentration of potassium). A two-site model best fit the data in either case but, in the presence of sodium, approximately twice as many sites were in the lower affinity state. Data are from a single experiment which was repeated with identical results.

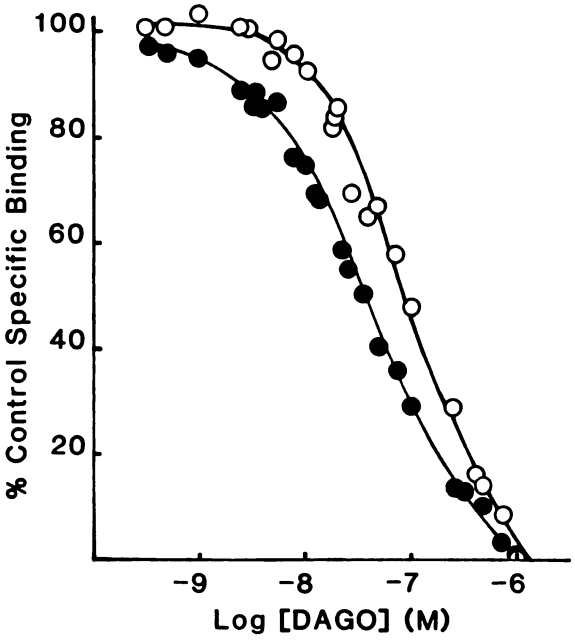

Fig. 5. Competition curve for DAGO against [^{3}H]DIP binding to μ sites with appropriate blockers present, as described in Materials and Methods, ○, in the presence of 120 mM sodium; ●, in the absence of sodium (sodium replaced by an equimolar concentration of potassium). A one-site model best fit the data in each case.

displacement of labeled antagonist under plus and minus sodium conditions. For these experiments we used DSLET as displacer since, over the concentration range used and under conditions which block its binding to other sites, this agonist is very selective for δ-type receptor sites. Although the antagonist was able to label two affinity states under both conditions, the proportion of higher affinity sites was about twice as great in the absence of sodium as in its presence. The total number of binding sites under each condition was the same. The lower affinity sites identified in these experiments cannot be identified by a labeled agonist, since the range of measurable binding above nonspecific falls below that necessary to characterize the lower affinity site. The 2-fold change in high affinity δ-binding site number induced by sodium in agonist and antagonist studies is particularly striking. It was also observed using labeled agonists both in NG108-15 cell delta receptors (6) and in δ-binding sites in guinea pig cortical membranes. This raises the possibility that sodium induces a receptor dimerization or polymerization, with resulting occlusion of half the binding sites. Recent reports of attempts to purify solubilized opioid receptors have provided some indications that opioid receptor proteins can associate in dimeric or higher-order polymeric forms (12). Enzymes composed of polymers of identical subunits sometimes display "half-of-the-sites-reactivity": that is, only half of the potentially available sites are occupied when the enzyme is saturated with ligand (13). It is also possible that the sodium-dependent reduction in the number of high affinity δ-binding sites is associated with the coupling of the receptor to a guanine nucleotide-binding protein, and perhaps the adenylate cyclase catalytic unit. The δ receptors in NG108-15 cells are functionally coupled to adenylate cyclase in a sodium-dependent manner (14), but it is not certain that δ-receptors in guinea pig cortical membranes are similarly coupled.

[^{3}H]EKC and [^{3}H]etorphine, under conditions which limited their binding to κ-receptors, both showed slight affinity changes in the presence of sodium, reflecting the lesser degree to which sodium regulates κ-type opioid binding. The relative insensitivity of κ binding in the absence of other cations (15, 16) and in

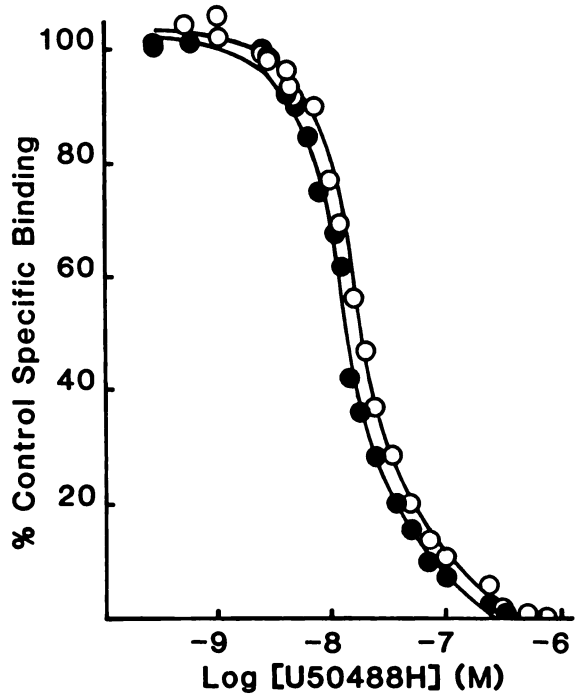

Fig. 6. Competition curve for U50,488H against [^{3}H]DIP binding to κ sites with appropriate blockers present, as described in Materials and Methods, (○) in the presence of 120 mM sodium; ●, in the absence of sodium (sodium replaced by an equimolar concentration of potassium). A one-site model best fit the data in each case.

their presence (8) has been reported. [^{3}H]DIP binding to κ-receptors was displaceable by the selective κ agonist U50,488H, with a K_i value slightly higher in the presence of sodium than in its absence.

The physiological role of the intracellular regulation by sodium of opioid agonist binding at μ and δ sites is not certain. In the preceding paper (6), in which we have studied cell lines carrying μ- or δ-receptors exerting an inhibitory control of adenylate cyclase, we have shown that the difference in the effect of sodium on agonist affinity is reflected in differences in the sodium regulation of opioid-inhibitory action. However, sensitivity to regulation by intracellular sodium need not imply that guinea pig cortical membrane μ- and δ-receptors are coupled to adenylate cyclase. Sodium may affect agonist affinity by regulating the association of guanine nucleotide-binding proteins with the receptors (17). However, our results do indicate that the mechanism of this interaction probably differs between μ- and δ-receptors. Since half-maximal effects of sodium are observed at concentrations comparable to intracellular sodium concentrations, it is possible that agonist affinity is modified by physiological and pharmacological factors influencing intracellular sodium. Agonist binding at κ sites was less sensitive to regulation by sodium, and there is less evidence that this action is mediated at an intracellular site.

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