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Repeated Electroconvulsive Shock: Effect on Sodium Dependency and Regional Distribution of Opioid-Binding Sites

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SUMMARY

The effects of single and repeated electroconvulsive shock (ECS) on the binding of [^{3}H]diprenorphine to rat brain membranes was studied. Repeated but not single ECS significantly increased the B_{max} of [^{3}H]diprenorphine binding when measured in the absence but not in the presence of NaCl. On a regional basis the effect of ECS was greatest in the olfactory bulb, nucleus accumbens, and striatum. More modest increases were found in the hippocampus, amygdala, septum, hypothalamus, and pyriform cortex. No significant effect was found in the brainstem and frontal cortex. Although the regional rank order of receptor increase does not match the receptor distribution of brain enkephalins, the receptor increase does parallel the regional increases in brain enkephalins following ECS.

The molecular mechanisms responsible for the short- and long-term behavioral effects of ECS remain enigmatic but most probably involve the interactions of several neurotransmitter and neuromodulator systems. In this regard, the brain opioid systems appear to have an important role. ECS has been shown to activate opioid systems within the brain (1). In rats, a single ECS produces a naloxone-reversible opioid-like catalepsy, analgesia, respiratory depression, baroreceptor reflex inhibition, and a slow wave synchronous EEG pattern (2-4). Repeated daily ECS results in a sensitization rather than a desensitization to the effects of morphine; similarly, chronic morphine administration enhances the opioid-like effects of acute ECS (5). Moreover, although opioid-like catalepsy and EEG effects become more protracted with repeated daily ECS, the ability of naloxone to reverse these effects diminishes over time (4).[2]

Repeated daily ECS treatment induces a significant increase in the B_{max} for DADLE binding to whole rat brain synaptic membranes (6). In contrast, a single ECS or repeated sham ECS induces no significant effects upon binding variables. These data were taken to indicate that opioid sensitization seen with repeated daily ECS may be associated with an increase in opioid receptor availability, especially of the δ receptor system.

The present study was undertaken to further clarify the changes in the brain's opioid receptor systems after repeated ECS. For these experiments, the "universal" opioid antagonist, [^{3}H]DP, was used as the primary receptor ligand. Recently, use was made of the ideal binding properties of [^{3}H]DP to characterize opioid receptor thermodynamics (7). The reasons for using [^{3}H]DP in the present studies were as follows. 1) The binding properties of [^{3}H]DP are such that one can easily obtain complete binding isotherms, including several data points at saturation. Furthermore, [^{3}H]DP binds as if the brain contained a single uniform population of high affinity (K_D~300 pM) sites. Thus, it is possible to determine directly, rather than indirectly as from Scatchard or computer analyses, changes in binding variables. 2) The high affinity and specificity of [^{3}H]DP binding make it an ideal ligand for use with small amounts of tissue (<100 μg of protein). This property was relevant to the present study since one of our goals was to examine the effects of repeated ECS in discrete brain regions.

Materials and Methods

Experimental procedures. Three groups (n = 12-18 rats/group) of male Sprague-Dawley rats (300-350 g; Zivic-Miller Laboratories) were evaluated. Group 1 received transauricular ECS (2 sec, 60 Hz, 50 mamp) daily for nine consecutive days; group 2 was given sham ECS (no current) for 9 days; group 3 received a single ECS on day 9. In some experiments, a fourth group was examined; these rats received a single ECS on day 1 only.

On the day following the last ECS, rats were decapitated. Two

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[2] F. C. Tortella, unpublished observation.

ABBREVIATIONS: ECS, electroconvulsive shock; DADLE, [^{3}H]-D-Ala^{2}D-Leu-enkephalin; DP, diprenorphine; BS, brainstem; FCx, frontal cortex; PCx, pyriform cortex; ST, striatum; HP, hippocampus; OB, olfactory bulb; NA, nucleus accumbens; MB, midbrain; HT, hypothalamus.

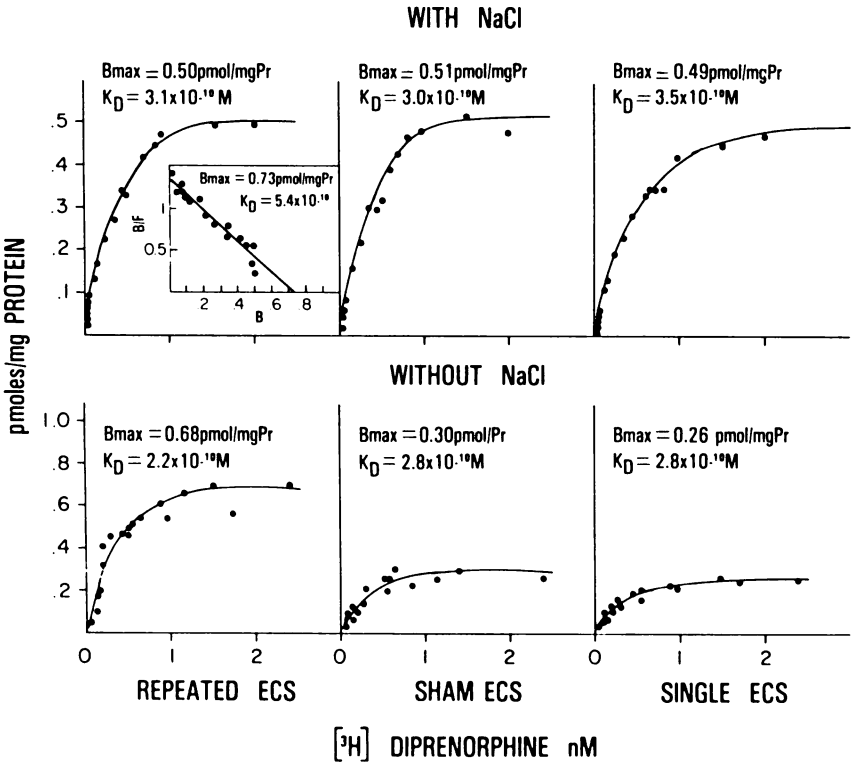

Fig. 1. Effect of acute and chronic ECS on [^{3}H]DP binding to rat brain. The chronic ECS group received transauricular ECS (2 sec, 60 Hz, 50 mamp) for nine consecutive days. The acute ECS group received a single ECS on day 9. The sham ECS group was given ECS (no current) for 9 days. On day 10, the animals were sacrificed, and brains were removed, washed, and immediately frozen at −70° in 0.32 M sucrose ± 20 mM Tris-HCl (pH = 8.0) until analysis. Membranes were prepared and binding performed as described by Hitzemann *et al.* (7). Typically, 20 concentrations of [^{3}H]DP ranging from 50 nM to 8 nM were employed (the 4 and 8 nM data are not shown in the figure). Data were analyzed by simple "pencil and paper" graphical techniques which were facilitated by obtaining several data points at saturation (B_{max}). The *upper three panels* show data obtained in the presence of 100 mM NaCl. The *inset* gives the Scatchard transformation of the data for chronic ECS + NaCl. Data in this figure are representative of a single experiment. Data summarizing all experiments are found in Table 1.

procedures were then followed. In one, brains minus cerebella were weighed, immediately immersed in 7.0 ml of ice-cold sucrose buffer (0.32 M sucrose, 10 mM Tris, pH = 7.96), and frozen on dry ice for shipment to R. J. H. for binding assays. For the other, the brains were rapidly dissected by J. L. M. into 11 brain regions, and the regions were individually wrapped in aluminum foil, frozen in dry ice, and then stored at −70° until shipment to R. J. H. Individual rat brains or brain regions were coded before shipment.

Membrane preparation. Whole rat brains or brain regions were thawed and homogenized in 19 volumes of cold sucrose buffer, and membranes were prepared (7). After preparation, endogenous opioids were removed from the membranes by a salting-out procedure (7). Radioreceptor assay of a 1 N acetic acid membrane extract confirmed that the salting procedure eliminated >98% of endogenous opioids.

Receptor binding assay. Binding assays were performed using the typical filtration assay (see, e.g., Ref. 7). All assays were performed at 37° with either 50 mM Tris, pH = 7.6, or 50 mM Tris and 100 mM NaCl as the binding buffer. The length of the binding assay was determined empirically for each brain region (or whole brain) by determining the equilibrium time for the lowest concentration of ligand employed. This time varied from 20 to 40 min. The concentration of [^{3}H]DP (specific activity 5 Ci/mmol) was varied from 20 pM to 8 nM. "Apparent" saturation was generally obtained by 1 nM. For each experiment, a minimum of 12 and a maximum of 20 concentrations of [^{3}H]DP were used. Typically, 50–100 μg of protein were used per assay. For the whole brain studies *individual* rat brains were used to construct saturation curves with each ligand. For the regional studies, pooled samples from control brains were used to construct saturation curves. Based on these data, determinations were made on individual brain regions, using ⅓, 1, and 3 times the empirically determined K_D concentrations. ECS-induced changes in K_D would diminish with increasing substrate concentration, whereas changes in B_{max} should remain relatively constant over the range studied.

Data analysis. The saturation isotherms obtained with the whole brain membranes were analyzed in four ways: 1) direct graphical examination, 2) conventional Scatchard analysis, 3) computer analysis using the ALLFIT program to generate Klotz plots (8), and 4) computer analysis using the LIGAND program (9). Methods 1, 3, and 4 gave virtually identical results, whereas the Scatchard analysis differed significantly (see Fig. 1). Although in theory the linear and nonlinear approaches should give equivalent results, errors in the determination of specifically bound ligand are amplified by the Scatchard approach, i.e., errors in duplicate measurements are largely along the radial direction in the plot of B/F versus B, indicating that, experimentally, the major source of error is in the determination of B. Since we can obtain complete binding isotherms (for [^{3}H]DP), we have avoided using the Scatchard interpretation of our results. LIGAND gave equivalent results to the Klotz plots and demonstrated a significantly better fit of all isotherms to a one- as opposed to a two- or three-site model. Hill numbers for all isotherms were not significantly different from 1 (actual range 0.94–1.06).

All data were analyzed by analysis of variance techniques, followed by t tests (two-tailed) when appropriate.

Results

[^{3}H]DP Binding

Whole brain. The data in Fig. 1 illustrate representative experiments for the effects of ECS on [^{3}H]DP binding in whole rat brain. The plot of bound versus free ligand revealed that saturation of binding occurred with all experimental groups. No further increase in binding was seen at 4 and 8 nM DP (data not shown). Scatchard plots (see Fig. 1, *inset*) of the data revealed no significant deviation from linearity, except at the high saturating concentrations. The B_{max} and K_D values obtained from the Scatchard-type analysis of the data were consistently higher than the values obtained from either the graphical (Fig. 1) or the semilogarithmic analyses (Table 1). Furthermore, the error (least square) of the Scatchard analyses was consistently greater than that of the semilogarithmic analyses for all data sets. Thus, the Scatchard type analysis was not employed in our evaluation of the experimental results.

The K_D and B_{max} values shown in Fig. 1 were determined by graphical analysis; a summary of the results from all experi-

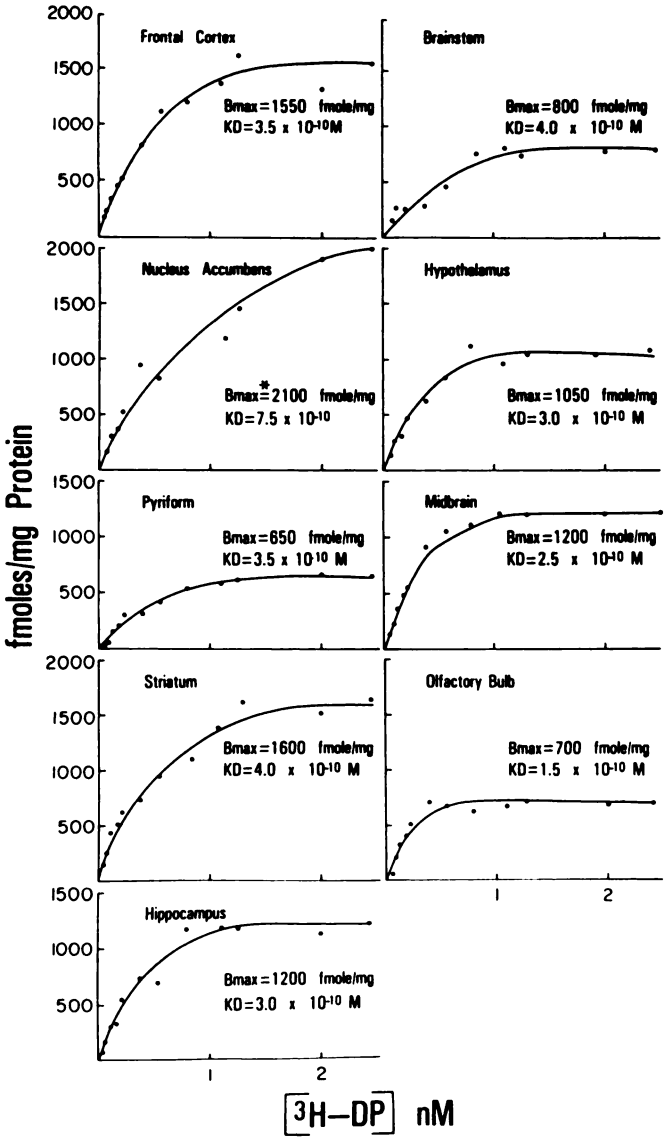

Fig. 2. [^{3}H]DP binding isotherms in discrete regions of the rat brain. After sacrifice, brains from sham ECS animals were immediately dissected and then frozen at −70°. Binding isotherms were constructed as in the legend to Fig. 1. Data are from the mean of three separate experiments.

ments is given in Table 1. In the presence of 100 mM NaCl (+NaCl), there was no significant difference among groups for K_D ($F_{2,15}$ = 1.68) or B_{max} ($F_{2,15}$ = 0.25). By contrast, in the absence of NaCl (−NaCl), there was a significant difference among groups ($F_{2,15}$ = 11.4) for B_{max} values, but not for K_D values ($F_{2,15}$ = 0.89). Repeated ECS treatment significantly ($p < 0.01$) increased the B_{max} in comparison to both the sham ECS group (+106%) and the single ECS group (+134%).

The ECS binding data were reanalyzed in the format suggested by Klotz (8) using the ALLFIT program. The data in Table 1 show the calculated K_D and B_{max} values ± standard errors for the same experiments shown in Fig. 1. For both the K_D and B_{max} values, the SE values ranged from 5 to 10%, illustrating the goodness of fit of the experimental data to the binding isotherm. As with the direct graphical analysis, there was a significant difference among the −NaCl groups ($F_{2,15}$ = 13.3) for B_{max} values. Using this method of data analysis, repeated ECS increased the B_{max} in comparison to the sham ECS group (+132%) and the single ECS group (+118%) (group data not shown).

Regional studies. Saturation isotherms in sham ECS animals for [^{3}H]DP binding were determined in the following brain regions: BS, FCx, PCx, ST, HP, OB, NA, HT, and MB. The results obtained for typical experiments are shown in Fig. 2; the B_{max} and K_D values presented are the mean of three such experiments. Significant regional heterogeneity in binding was found. The order of binding affinity was OB > MB = PCx, HT, HP, ST, BS, FCx > NA. The order of binding density was NA > ST = FCx > HP = MB = HT > BS = OB = PCx. Binding density in the NA was 323% greater than in the PCx. In two brain regions, the septum and the amygdala, it was not possible to accurately determine the binding isotherms because of the low density of receptor sites. The K_D for both regions appeared to be approximately 3×10^{-10} M and the B_{max} for these regions was less than 100 fmol/mg of protein.

A comparison [^{3}H]DP binding in the discrete brain regions among the experimental groups was made using ⅓, 1, and 3 times the empirically determined K_D values. For these experiments, an additional ECS group which received a single ECS on day 1 was included in the analyses. The results are summarized in Table 2. Analysis of variance statistics were used to detect significant group differences. Data are presented as a percentage of the sham ECS control. No significant treatment effect was found in the FCx. The OB showed the most marked increase in binding after chronic ECS ranging from +72% at the lowest ligand concentration (50 pM) to +57% at the highest concentration (4.50 pM). Changes of a similar magnitude and

TABLE 1

Effects of sham, single, and chronic ECS on the binding of [^{3}H]DP to whole rat brain membranes

Rats received sham, single, or chronic ECS as described in Materials and Methods. Twenty-four hr after the final treatment, animals were sacrificed and brain membranes were prepared. [^{3}H]DP binding was measured as described by Hitzemann *et al.* (7). K_D and B_{max} values were determined by graphical analysis or by the method of Klotz (8) using the ALLFIT program (values in parentheses). Data are the mean ± standard error of six to nine separate experiments.

Group[a]	K_D		B_{max}	
	−NaCl	+NaCl	−NaCl	+NaCl
	10^{-10} M		pmol/mg of protein	
Sham ECS	2.2 ± 0.4 (2.07 ± 0.45)	3.6 ± 0.4 (4.03 ± 0.56)	0.31 ± 0.03[a] (0.28 ± 0.02)[a]	0.54 ± 0.06 (0.53 ± 0.04)
Single ECS	2.8 ± 0.4 (3.09 ± 0.15)	3.2 ± 0.5 (3.37 ± 0.31)	0.33 ± 0.04[a] (0.29 ± 0.06)	0.51 ± 0.06 (0.54 ± 0.03)
Chronic ECS	1.9 ± 0.2 (2.14 ± 0.25)	2.4 ± 0.4 (3.32 ± 0.36)	0.72 ± 0.08[b] (0.67 ± 0.03)	0.58 ± 0.09 (0.57 ± 0.03)

[a] Significantly different from +NaCl value, $p < 0.01$.
[b] Significantly different from sham ECS value, $p < 0.01$.

TABLE 2

Effect of single and chronic ECS on [^{3}H]DP binding to discrete regions of the rat brain

The effects of single ECS (day 1 or day 9) and chronic ECS on [^{3}H]DP binding were determined in discrete regions of the rat brain at ⅓, 1, and 3 times the empirically determined K_D values for the various regions (see Fig. 3). Data are expressed as mean per cent of the sham ECS value ± standard error. Data are the mean of six separate experiments.

Brain region	[DP]	Percentage of sham ECS values		
		Single (day 1)	Single (day 9)	Chronic (days 1–9)
	M			
Hippocampus	1.0×10^{-10}	114 ± 13	121 ± 8[a]	148 ± 11[b]
($K_D = 3.0 \times 10^{-10}$ M)	3.0×10^{-10}	88 ± 9	102 ± 3	139 ± 9[b]
	9.0×10^{-10}	97 ± 6	84 ± 14	131 ± 10[a]
Striatum	1.3×10^{-10}	89 ± 9	111 ± 5	162 ± 13[b]
($K_D = 4.0 \times 10^{-10}$ M)	4.0×10^{-10}	91 ± 8	118 ± 11	145 ± 9[b]
	12.0×10^{-10}	109 ± 14	89 ± 13	141 ± 13[b]
Olfactory bulb	0.5×10^{-10}	116 ± 9	108 ± 5	172 ± 13[b]
($K_D = 1.5 \times 10^{-10}$ M)	1.5×10^{-10}	128 ± 7[a]	92 ± 5	165 ± 18[b]
	4.5×10^{-10}	119 ± 12	83 ± 11	157 ± 14[b]
Nucleus accumbens	2.5×10^{-10}	91 ± 12	108 ± 7	152 ± 13[b]
($K_D = 7.5 \times 10^{-10}$ M)	7.5×10^{-10}	103 ± 9	111 ± 13	142 ± 9[b]
	22.5×10^{-10}	111 ± 7	86 ± 9	162 ± 15[b]
Amygdala	1.0×10^{-10}	NA[c]	NA	NA
(ca. 3.0×10^{-10} M)	3.0×10^{-10}	118 ± 9	122 ± 7[a]	132 ± 11[a]
	9.0×10^{-10}	114 ± 7	118 ± 11	146 ± 9[b]
Midbrain	0.8×10^{-10}	84 ± 7	109 ± 6	143 ± 6[b]
($K_D = 2.5 \times 10^{-10}$ M)	2.5×10^{-10}	102 ± 13	112 ± 6	129 ± 11[a]
	7.5×10^{-10}	100 ± 12	105 ± 9	133 ± 8[a]
Brainstem	1.3×10^{-10}	109 ± 11	113 ± 16	130 ± 8[a]
($K_D = 4.0 \times 10^{-10}$ M)	4.0×10^{-10}	86 ± 7	109 ± 7	121 ± 11
	12.0×10^{-10}	95 ± 13	115 ± 12	118 ± 9
Septum	1.0×10^{-10}	NA	NA	NA
($K_D = 3.0 \times 10^{-10}$ M)	3.0×10^{-10}	121 ± 11	125 ± 7[a]	141 ± 7[b]
	9.0×10^{-10}	118 ± 13	120 ± 11	128 ± 13
Hypothalamus	1.0×10^{-10}	101 ± 12	114 ± 9	131 ± 11[a]
($K_D = 3.0 \times 10^{-10}$ M)	3.0×10^{-10}	113 ± 8	106 ± 3	142 ± 9[b]
	9.0×10^{-10}	113 ± 11	109 ± 11	147 ± 8[b]
Pyriform cortex	1.2×10^{-10}	101 ± 8	114 ± 13	138 ± 11[a]
($K_D = 3.5 \times 10^{-10}$ M)	3.5×10^{-10}	111 ± 7	109 ± 5	142 ± 7[b]
	10.5×10^{-10}	93 ± 11	98 ± 11	149 ± 12[b]
Frontal cortex	1.2×10^{-10}	97 ± 11	109 ± 8	121 ± 10
($K_D = 3.5 \times 10^{-10}$ M)	3.5×10^{-10}	108 ± 9	117 ± 9	123 ± 12
	10.5×10^{-10}	111 ± 14	113 ± 7	118 ± 11

[a] Significantly different from sham ECS, $p < 0.05$.
[b] Significantly different from sham ECS, $p < 0.01$.
[c] NA, not applicable.

pattern were found in the ST and NA. Somewhat more modest but significant increases after chronic ECS were found in the HP, HT, MB, PCx, and amygdala. Two brain regions, the BS and septum, showed significant effects of chronic ECS at only one substrate concentration. Acute (single) ECS treatment had no consistent effect on [^{3}H]DP binding.

Discussion

Repeated daily ECS both sensitizes animals to the opioid-like behavioral and EEG effects of acute ECS and attenuates the ability of naloxone to reverse these effects (10).[3] These data contrast with the effects of chronic opioid administration, namely, tolerance and increasing naloxone sensitivity (11). Thus, chronic ECS may be viewed as inducing a "reverse tolerance" in at least some of the brain's opioid systems. In an attempt to understand the molecular mechanism(s) of this phenomenon, we have focused our investigations on the effects of repeated ECS on the opioid recognition (binding) sites. Recent studies point to the plasticity of these sites. For example, up-regulation of opioid binding has been reported following chronic antagonist administration (12). Although down-regulation has not been observed after chronic *in vivo* opioid treatment, impressive agonist effects have been found *in vitro*. Dingledine *et al.* (13) have shown that acute desensitization to the excitatory effects of DADLE in rat hippocampal slices is coupled with a decrease in δ binding sites. Interestingly, desensitization to the effects of the μ agonist, morphiceptin, did not alter the number of μ binding sites, suggesting significant differences in μ and δ receptor regulation. Confirming this view, Lenoir *et al.* (14) found that DADLE and etorphine, but not morphine, induced a down-regulation of [^{3}H]DP binding to rat brain fetal cell aggregates.

Based on these data, one could make some predictions regarding how ECS, which is known to release endogenous enkephalins in discrete brain regions (15), would affect opioid receptor binding. Repeated ECS could be expected to down-regulate δ binding sites while probably having no significant

[3] F. C. Tortella, unpublished observation.

effect on μ receptor binding. However, the results obtained in this and our previous study (6) clearly demonstrate that chronic ECS specifically *increases* the receptor binding of at least two opioid ligands, DP and DADLE. This reverse of the expected desensitization is, of course, consistent with the behavioral observations of reverse tolerance. Furthermore, these data support the behavioral evidence implicating both the δ receptor system and the antagonist receptor conformation in the mechanisms of chronic ECS. Finally, it should be noted that, in preliminary studies, we have been unable to detect any specific effects of ECS on [^{3}H]morphine binding; these results further illustrate the apparent lack of plasticity in μ recognition sites.

The advantage of using [^{3}H]DP to quantify the increase in binding sites after chronic ECS was easily realized. The increase in B_{max} was determined directly (Fig. 1) but was also confirmed by a computer-based fitting program (Table 1). Thus, although the significance of the increase in [^{3}H]DP binding is still hypothetical, this phenomenon seems clearly established. The increase in [^{3}H]DP binding is found only in the absence of NaCl. Inasmuch as the "Na^+ effect" is not well understood at the molecular level, it is probably unwise at this time to view the increase as an up-regulation or mobilization of spare or cryptic receptors.

The regional studies clearly demonstrate that the increase in [^{3}H]DP seen in the absence of NaCl is not nonspecific. Although the regional rank order of receptor increase does not match the receptor distribution (Fig. 2) or the regional distribution of brain enkephalins, the receptor increase does parallel the regional increases in brain enkephalins following ECS (15). The large increase in the olfactory bulb may signal that this brain region, with its relatively simple architecture, is more deserving of our attention. It is perhaps more than coincidental that the three brain regions which showed the largest receptor increase, the OB, NA, and ST, are particularly rich in enkephalinergic systems (16).

The increase in binding sites is significant only if these sites are functionally linked to the other elements of the receptor complex. The behavioral data clearly suggest that sensitivity to both exogenous and endogenous opioids changes after chronic ECS and an increase in functional receptors would be a parsimonious explanation for these data. Since the increase occurs in one brain region, the striatum, where the link between the opioid-binding sites and the adenylcyclase system has been established (17), there is the potential of testing whether or not the binding sites are functionally coupled (18). In conclusion, the present study confirms and extends our earlier observation (6) that chronic ECS specifically increases brain opioid receptor binding. The increase in [^{3}H]DP shows a regional specificity and is likely to involve a change in the mechanism(s) by which Na^+ regulates receptor site availability.

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