

ACUTE SYSTEMIC ADMINISTRATION OF MORPHINE SELECTIVELY INCREASES MU OPIOID RECEPTOR BINDING IN THE RAT BRAIN

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Opioid receptor binding, including the mu, delta, and kappa receptor subtypes, was compared in morphine-injected and control rats. Brain tissues were homogenized and centrifuged either one or two times prior to receptor binding assay. In brain membranes from morphine-injected rats centrifuged once, there was a decrease in mu, but not delta or kappa, binding compared to controls, perhaps indicating occupation of these sites by morphine. By contrast, homogenates from morphine-injected rats centrifuged twice manifested an increase in mu, but not delta or kappa, binding sites. These results suggest that pharmacological stimulation of central opioid receptors with morphine causes a rapid, selective increase in the number of available mu binding sites.

INTRODUCTION

It is now widely held that multiple subtypes of opioid receptors mediate the various physiological and behavioral effects of endogenous and exogenous opioids (for recent reviews see 1,2). A common theme in the description of multiple receptor subtypes is the attempt to ascribe particular functions to specific receptors (e.g. 3,4). Converging lines of evidence, from a variety of in vitro bioassays, receptor binding assays, and in vivo pharmacological studies, points to an important role for the mu receptor in mediation of analgesia (e.g. 5-7). There are, however, reports that kappa and delta selective agents can also induce analgesia depending upon variables such as the route of administration and the type of pain inhibited (e.g. 7). In the present study, we have shown that morphine, the prototypic opiate analgesic and mu receptor agonist, selectively occupies mu opioid receptors in vivo following administration of pharmacologically relevant doses. Interestingly, following systemic administration of analgesic doses of morphine, a pronounced increase in the number of mu, but not delta or kappa, binding sites was observed.

METHODS

Subjects were male Sprague-Dawley rats (200-250g; Charles River Laboratories, Worcester, MA). Rats were injected subcutaneously with either morphine sulfate (5 mg/kg) or 0.9% saline 20 min prior to sacrifice by decapitation. Brains (minus cerebellum) were rapidly removed, placed in ice cold Tris buffer (50 mM, pH 7.4), and homogenized with a Brinkman Polytron.

Final tissue concentration was 50 mg wet weight/ml Tris. Homogenates were then centrifuged (15 min, 27,000 x g) and the pellet resuspended in Tris buffer. Subsequently, half of each homogenate was centrifuged a second time in order to wash the tissue further prior to the binding assay. The receptor binding was carried out in a volume of 0.25 ml comprising 0.05 ml [<sup>3</sup>H]-ligand (concentrations ranging from 0.5-40 nM), 0.050 ml of Tris or 2 micromolar unlabelled ligand (to determine total and non-specific binding, respectively), and 0.15 ml of brain homogenate. Binding of each of the opioid receptor subtypes was defined using [<sup>3</sup>H]-D-Ala<sup>2</sup>-MePhe<sup>4</sup>-enkephalin-gly<sup>15</sup> (DAGO; RX783006, Amersham, 30 Ci/mmol) for mu binding, [<sup>3</sup>H]-D-Ser<sup>2</sup>-D-Thr<sup>6</sup>-Leucine enkephalin (DSLET; New England Nuclear, 30.5 Ci/mmol), and [<sup>3</sup>H]-bremazocine (New England Nuclear, 30.5 Ci/mmol) in the presence of 100 or 1000 nM unlabelled DAGO and DSLET for kappa binding (8). The receptor assay mixture was incubated on ice for 90 min in 96 well microtiter plates, filtered under vacuum on Schleicher and Schuell #32 glass fiber filters using a Brandel cell harvester, and the filters counted in a liquid scintillation counter.

Data for analysis were obtained in two ways. For production of Scatchard plots, brains of three rats in each injection condition were pooled and the homogenate was assayed with radiolabelled ligands in concentrations ranging from 0.05 nM to 40 nM. Data from this experiment were analyzed using the computer program LIGAND (9) to obtain best fit estimates of the binding parameters  $K_D$  and  $B_{max}$ . For statistical comparison of the observed differences in binding between saline and morphine-injected groups, brains of individual rats from each group (n=6) were assayed with 0.5 and 5.0 nM of each of the [<sup>3</sup>H] ligands, and following one or two centrifugations. Group differences were tested using analysis of variance with repeated measures and Newman-Keuls tests for specific comparisons. A significance level of  $p < .01$  was used for all inferential statistics.

## RESULTS

Morphine caused an apparent decrease in the binding capacity of brain membranes for the mu, but not the delta or kappa, ligands (Table 1) indicated by the observed decrease in the apparent  $K_D$  after one centrifugation. That this difference represents receptor occupation by morphine is consistent with classically defined competitive binding models. There was, however, an accompanying increase in  $B_{max}$ . After the second centrifugation, membranes from morphine-injected brains showed a marked increase in the total number of mu, but not delta or kappa, binding sites (Table 1). In this case, the apparent  $K_D$ s for [<sup>3</sup>H]-DAGO binding are similar in control and morphine-injected homogenates suggesting successful elimination of bound morphine and the remaining effect being a marked increase in  $B_{max}$ .

Analysis of the data from animals assayed individually revealed the reduction of in vitro binding to be mu selective and statistically reliable. Moreover, the apparent increase mu binding in membranes centrifuged twice from morphine-injected rats was also significant and specific to the mu receptor.

Table 1. EFFECT OF MORPHINE (5 mg/kg) ON OPIOID RECEPTOR BINDING.

		K <sub>D</sub> (nM)			B <sub>max</sub> (fmoles/mg)		
		CON	MOR	%CON	CON	MOR	%CON
MU	1 SPIN	1.3+.1	2.1+.3	162	13.2+.4	16.4+.1	124
	2 SPINS	1.1+.1	1.2+.1	109	11.3+.5	15.1+.4	134
DELTA	1 SPIN	3.1+.4	3.1+.5	100	12.7+.8	11.8+.9	93
	2 SPINS	3.4+.3	3.8+.3	112	11.4+.5	12.9+.6	113
KAPPA*	1 SPIN	.4+.05	.4+.03	100	2.2+.1	2.1+.1	95
	2 SPINS	.4+.03	.4+.03	100	1.9+.1	1.9+.1	100

Data presented are best fit parameter estimates + S.E.M derived from the LIGAND program. Control=CON, Morphine-injected=MOR, Percent of Control=%CON.  
 \* Analysis of [<sup>3</sup>H]-Bremazocine binding indicated the presence of 2 sites. These data represent only the high affinity site.

#### DISCUSSION

Examination of receptor binding properties of brain tissue from morphine-injected rats following homogenization and either one or two centrifugations illustrates two points. First, when binding is done in tissue centrifuged once, to remove endogenous opioids liberated by homogenization and non-receptor associated morphine, there appears to be a significant occupation of mu, but not delta or kappa, sites. After the second centrifugation, which presumably washes off receptor-bound morphine, it is revealed that systemic administration of morphine appears to have caused a rapid, substantial (approximately 35%), and selective increase in brain mu opioid receptors. The present results, by the use of selective [<sup>3</sup>H]-ligands, confirm and extend the original observation of this phenomenon by Pert and Snyder (10).

In recent work attempting to elucidate the mechanism of this apparent increased number of mu receptors we have made the following observations: 1) This phenomenon is general in that it occurs in spinal cord as well as brain, but is specific in that it appears not to be elicited by non-mu opioid agonists (e.g. the kappa selective drug U-50,488H). 2) In vivo labelling of mu receptors by systemic administration of [<sup>3</sup>H]-etorphine (10-40 micro-Ci/kg; see 6) is enhanced by co-administration of a low dose of morphine suggesting increased receptor availability. 3) The effect is rapid in onset (10 min; see also reference 10), dose dependent (maximal at 10-20 mg/kg), and of prolonged duration (maximal at 1 hr and still significant up to 3 hr after morphine injection).

Several hypotheses which could account for the apparent receptor-inducing effects of morphine. It may be that many mu receptors are tonically occupied by an endogenous ligand that is displaced, in vivo, by morphine. Perhaps this ligand, present on the receptors of saline-injected animals and not as easily eliminated as morphine during the brain homogenate preparation, remains throughout the binding assay effectively masking mu sites. Thus, more mu receptors may be available to the [<sup>3</sup>H]-ligands during the assay in membranes from morphine-injected rats. Alternatively,

stimulation of opioid systems by morphine may cause the activation of normally inert receptors; possibly via insertion of soluble receptors into membranes or enzymatic activation of dormant receptors (e.g. 11). Although many questions remain regarding the underlying physiological mechanisms and behavioral significance of this phenomenon, taken together our results suggests that systemic administration of opiates causes profound alterations in the binding characteristics of central nervous system opioid pathways.

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