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Comparison of Regional CNS Ligand Binding in Two Inbred Rat Strains: Effects of Chronic Morphine

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COOPER, D. O., K. R. CARLSON AND J. W. McKEARNEY. *Comparison of regional CNS ligand binding in two inbred rat strains: Effects of chronic morphine.* PHARMACOL BIOCHEM BEHAV **23**(3)349–354, 1985.—Male rats of the F-344 and BUF inbred strains were given free access to a 10% sucrose solution containing 0.5 mg/ml morphine sulfate (controls received sucrose only) as their sole source of fluids. The daily intake of morphine averaged 101 ± 13 mg/kg. After 18 days on this regimen, animals were sacrificed and assayed for ^{3}H-clonidine (alpha-2 adrenergic), ^{3}H-dihydroalprenolol (DHA, beta 1 and 2 adrenergic) and ^{3}H-spiperone (SPD, 5-HT$_2$ and D$_2$) binding in several brain regions. In the absence of morphine treatment, BUF rats displayed higher levels of SPD binding in brainstem, as compared with the F-344 strain. In contrast, untreated F-344 rats exhibited higher levels of DHA binding in hypothalamus and SPD binding in striatum than BUF rats. Chronic morphine resulted in an increase in clonidine and DHA binding in the brainstem and hippocampus respectively of BUF, but not F-344 rats, suggesting a greater sensitivity of adrenergic function to opiate treatment in the BUF strain. The two strains differed qualitatively in the effect of morphine on striatal SPD binding, with BUF rats exhibiting a decrease, and F-344 rats an increase. The one consistent change observed in both strains was a quantitatively similar increase in hippocampal SPD binding after chronic morphine. The results demonstrate that despite strain-dependent differences in binding characteristics, chronic morphine elicits a strain-independent alteration in hippocampal 5-HT$_2$ binding. On the basis of these preliminary findings, it may be speculated that this particular neurochemical consequence contributes to morphine-induced behaviors which are observed independent of rat strain.

Receptor binding Strain differences Chronic morphine

INTERACTIONS of opiate agonists and antagonists with noradrenergic (NA) and serotonergic (5-HT) mechanisms in the central nervous system have been studied extensively, but there are many discrepancies among published reports (for reviews see [5,27]). A similar lack of consistency is apparent in studies attempting to modify the behavioral manifestations of narcotic action through pharmacologic manipulation of NA [27] and 5-HT [5] function. Discrepancies have been attributed to differences in either species/strain or pharmacologic/biochemical methodology.

Inbred strains of rats and mice may exhibit considerable diversity in both behavioral (e.g., [4,16]) and neurochemical (e.g., [10, 28, 40]) characteristics. While these strain differences add complexity, they have also provided a useful tool for investigation of correlations between behavioral and biochemical parameters (e.g., [47,49]). In this regard, two inbred strains of mice (DBA and C57) have been shown to differ in locomotor and analgetic responses to morphine [43]. Furthermore, these same mouse strains display differential development of tolerance to morphine and d-ala-met-enkephalin [14]. These strain differences in behavioral response to opiates have been correlated with variations in striatal dopamine metabolism [6, 9, 43, 51], cyclic nucleotide disposition [43] and opiate receptor density [44].

Differences between inbred mouse strains in opiate receptor binding characteristics have been found by several investigators [1,45]. The potential significance of these variations to observed behavioral and biochemical differences following opiate administration has not, however, been thoroughly investigated. There are also a number of recent studies demonstrating differences in alpha-2 adrenergic [40] and dopaminergic [11,18] binding between inbred rat strains (Fisher F-344 and Buffalo BUF). Alterations in alpha-2 [15] and beta-adrenergic [15, 30, 31] binding have been reported following chronic morphine administration. Changes in serotonergic binding after chronic morphine have also been demonstrated, however, these studies were performed utilizing ^{3}H-5-HT, a radioligand which is known to preferentially label 5-HT$_1$ sites [39]. To our knowledge, the present experiments are unique in their assessment of 5-HT$_2$ binding following chronic opiate administration. Furthermore, none of the studies examining binding alterations in response to opiate treatment have evaluated possible strain differences.

Observed discrepancies in the literature concerning alterations in other neurochemical parameters (i.e., neurotransmitter levels and turnover) following morphine treatment suggest that strain-dependent differences in ligand binding may appear in conjunction with long-term morphine exposure. This paper describes preliminary results which indicate that rats of the F-344 and BUF inbred strains differ

significantly in certain adrenergic and serotonergic binding characteristics and in the alterations induced by chronic morphine treatment.

METHOD

Male F-344 (Charles River) and BUF (Microbiological Associates) rats were randomly assigned to control and experimental groups (N=5 per group). The animals weighed 250–300 g at the onset of experimentation and were housed individually in a climate controlled room with an 0700 on–1900 off light cycle. Subjects in the experimental groups were given free access to Purina rat chow and to a 10% sucrose solution containing 0.5 mg/ml morphine sulfate (Mallinckrodt) as their sole source of fluids. This procedure has been shown to induce physical dependence in as little as 5 days [29]. Control animals were given free access to food and 10% sucrose vehicle. Subjects were maintained on this regimen for 18 days, during which time no significant differences between strains were observed in morphine intake. The average daily self-administered dose was 101±13 mg/kg.

On the 19th day all animals were sacrificed by decapitation and brains were quickly removed and dissected over ice into frontal cortex, hippocampus, striatum, hypothalamus and brainstem (pons-medulla). Brain regions were frozen in aluminum foil on dry ice and kept at −70°C until assay. Assays for clonidine [52], DHA [7] and 5-HT_2/D_2 [48] binding were carried out according to established protocols with minor modification.

Tissue was homogenized (Brinkmann Polytron, setting 7, two 5 second bursts) in ice-cold 50 mM Tris buffer, pH 7.4 (hereinafter referred to as Tris) at a concentration of 10 mg wet weight per ml (resulting in approximately 1 mg protein per assay tube). The homogenate was then centrifuged at 48,000×g for 10 minutes and the supernatant was discarded. The pellet was resuspended in a fresh equal volume of Tris and rehomogenized and re-centrifuged as above. The final pellet was once again resuspended and rehomogenized for assay.

Binding assays were performed in duplicate in 12×75 mm glass culture tubes. Duplicate total binding tubes contained 0.1 ml radioligand and 0.1 ml Tris. Non-specific binding tubes contained 0.1 ml radioligand and 0.1 ml of the appropriate displacing agent. Ligands and displacing agents for each type of assay were (1) alpha-2 adrenergic: ^{3}H-clonidine (S.A. 5.28×10^{13} dpm/mMol, New England Nuclear, NEN) final concentration 3.0 nM displaced by levarterenol 0.5 mM; (2) beta-adrenergic: ^{3}H-dihydroalprenolol (DHA, S.A. 1.09×10^{14} dpm/mMol, NEN) 1.0 nM displaced by dl-propranolol 1.0 μM; (3) 5-HT_2/D_2: ^{3}H-spiperone (SPD, S.A. 5.28×10^{13} dpm/mMol, NEN) 0.3 nM displaced by d-LSD 1.0 μM.

Binding assays were begun by addition of 0.8 ml tissue suspension to prepared total and non-specific binding tubes over ice. After mixing, the tubes were allowed to incubate for 30 minutes at 22°C (clonidine and DHA) or for 15 minutes at 37°C (SPD). Following incubation, the contents of the tubes were poured over Whatman GF/B filters (pre-wetted with 2 ml Tris) under vacuum. The filters were then washed with 5 ml Tris. Incubation tubes were filled with 3 ml Tris and emptied once again over the filters. The filters received a final 5 ml Tris wash and were placed in 10 ml scintillation counting fluor (Ready-solv, Beckman). Filters were allowed to resorb in the counting fluid for 48 hours, after which they were counted with ^3H_2O standards to assess countereffiiciency (average 32%). Results were expressed as fmol of specific binding (total minus non-specific) ±S.E.M. per mg protein as assessed by the method of Lowry [35] using a bovine serum albumin standard curve. Each binding assay was replicated in three separate experiments incorporating all four groups.

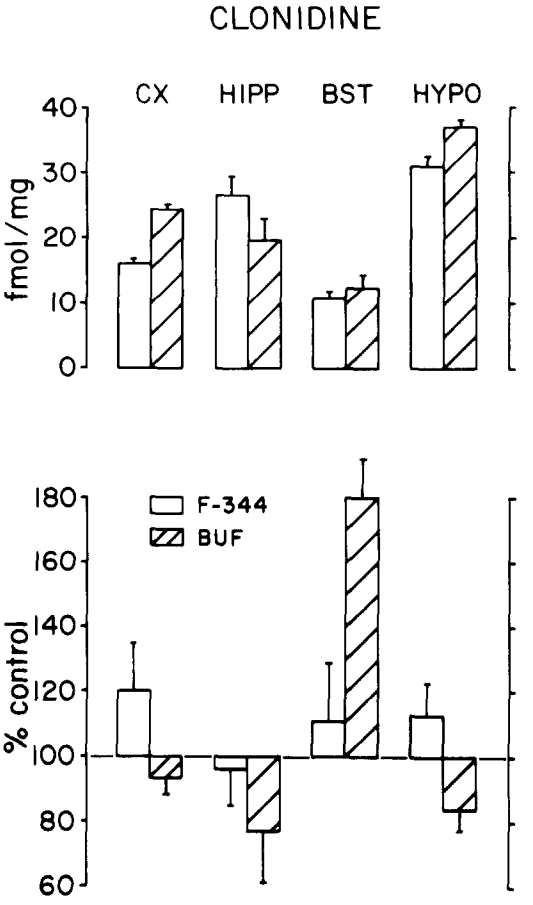

FIG. 1. ^{3}H-clonidine binding. Upper panel represents control levels of clonidine binding expressed in fmol/mg protein ± S.E.M. in frontal cortex (CX), hippocampus (HIPP), pons-medulla (BST) and hypothalamus (HYPO) of Fisher (F-344) and Buffalo (BUF) rats given free access to 10% sucrose only. Lower panel demonstrates alterations in clonidine binding expressed as percent of control in rats of both strains given free access to 10% sucrose containing 0.5 mg/ml morphine sulfate.

While saturation curves to derive information concerning the relative contributions of Bmax and Kd would have been valuable, the available tissue volumes were sufficient only for assessment of all three ligands at one concentration making Scatchard analyses impossible. Experiments are in progress to further characterize the nature of significant binding alterations which were observed.

Data for each ligand in each brain region were analyzed by two-way analysis of variance (strain×drug). Significant F values ($p<0.05$; $df=1,16$) presented in the results were further analyzed by the post-hoc Neuman-Keuls test to determine which individual comparisons were responsible for overall significance.

RESULTS

The data are presented by ligand, with the effects of mor-

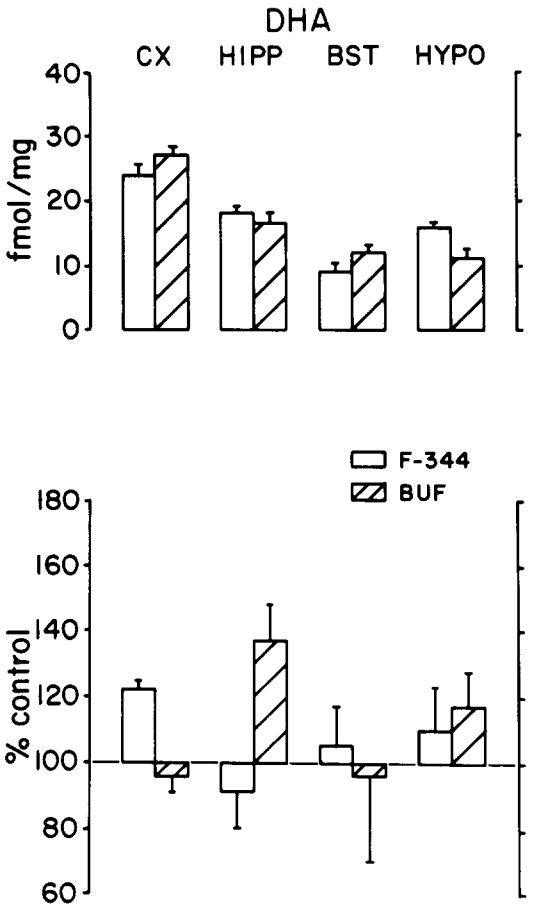

FIG. 2. ^{3}H-dihydroalprenolol binding. Upper panel represents control levels of DHA binding expressed as fmol/mg protein±S.E.M. in frontal cortex (CX), hippocampus (HIPP), pons-medulla (BST) and hypothalamus of Fisher (F-344) and Buffalo (BUF) rats given free access to 10% sucrose only. Lower panel demonstrates alterations in DHA binding expressed as percent of control in rats of both strains given free access to 10% sucrose containing 0.5 mg/ml morphine sulfate.

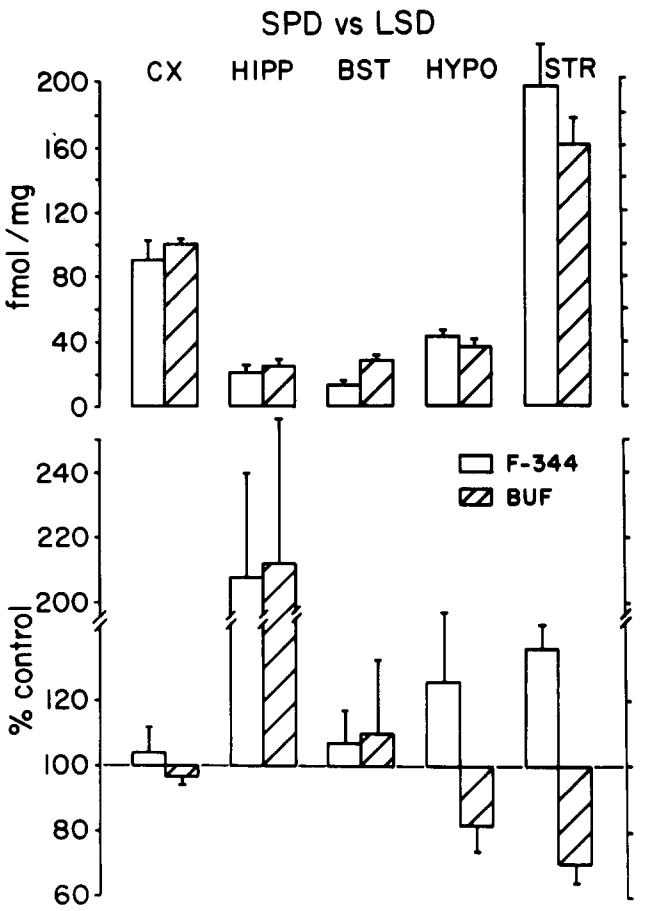

FIG. 3. ^{3}H-spiperone binding. Upper panel represents control levels of SPD binding expressed in fmol/mg protein±S.E.M. in frontal cortex (CX), hippocampus (HIPP), pons-medulla (BST) and hypothalamus (HYPO) of Fisher (F-344) and Buffalo (BUF) rats given free access to 10% sucrose only. Lower panel demonstrates alterations in SPD binding expressed as percent of control in rats of both strains given free access to 10% sucrose containing 0.5 mg/ml morphine sulfate.

phine (as percent of control) underneath baseline (control) levels of binding in various regions for both rat strains.

Baseline clonidine binding (Fig. 1, upper panel) was higher in hypothalamus ($F=4.85$) and frontal cortex ($F=12.63$) of BUF as compared with F-344 rats. Following chronic morphine (Fig. 1, lower) binding tended to increase in F-344 and decrease in BUF rats. The only effect which achieved statistical significance, however, was the substantial elevation of brainstem clonidine binding observed in the BUF strain (80%, $F=11.45$).

Baseline DHA binding (Fig. 2, upper) was regionally similar in both rat strains except in the hypothalamus, where F-344 rats displayed a higher level of binding than BUFs ($F=9.04$). Chronic morphine (Fig. 2, lower) elevated DHA binding in the hippocampus of BUF rats (37%, $F=5.70$). The apparent increase in frontal cortex of F-344s (Interaction, $F=5.58$) did not achieve significance in the Neuman-Keuls test.

Baseline SPD binding (Fig. 3, upper) was twofold higher in brainstem of BUF rats as compared with F-344s ($F=19.30$). In addition, baseline binding of SPD was significantly lower (23%) in the striatum of BUF as compared with F-344 rats. Interestingly, chronic morphine (Fig. 3, lower) reduced striatal SPD binding in BUF rats by a further 29%, while F-344s displayed a 36% increase in binding (Strain effect, $F=63.99$; Interaction, $F=24.45$; Neuman-Keuls $p<0.05$ for all comparisons).

A similar effect of morphine was observed in the hypothalamus, with F-344 rats again displaying an increase (26%) and BUF rats a decrease (18%) in SPD binding (Strain Effect, $F=7.57$), however, the individual drug effects failed to achieve significance in Neuman-Keuls analysis. The most striking morphine-induced alteration was a quantitatively similar increase in hippocampal SPD binding observed in both F-344 and BUF rats (108% and 112% respectively, Drug effect, $F=12.53$).

DISCUSSION

Rats of the BUF and F-344 strains differ considerably with respect to certain neurochemical parameters. In agreement with a previous investigation [52], the highest level of

clonidine binding was observed in the hypothalamus of both BUF and F-344 rats. The results also confirm a prior study demonstrating higher levels of clonidine binding in the hypothalamus of BUF, as compared with F-344 rats [40]. On the other hand, our finding of higher clonidine binding in the frontal cortex of BUFs as compared with F-344 rats differs from two previous studies demonstrating either no difference between strains [40] or higher clonidine binding in frontal cortex of F-344s [19]. The reasons for these apparent discrepancies may lie in the use of different ligands (e.g., para-amino-clonidine, [40]) or in varying biochemical procedure.

Given the large number of reports demonstrating alterations in norepinephrine metabolism following chronic morphine exposure, changes in adrenergic receptor regulation might be expected. We observed an increase in clonidine binding in the brainstem, and DHA binding in the hippocampus of BUF rats. There have been other reports of increases in brainstem clonidine binding after chronic morphine [15], but decreases have also been reported [50]. There is also a lack of consistency concerning alterations in beta-adrenergic binding (DHA) after chronic morphine. Some investigators report increases in DHA binding in cortex and brainstem of dependent rats [34], which is rapidly reversed during naloxone precipitated withdrawal [15]; others report no alterations in DHA binding in dependent rats, but increases following naloxone administration [30,31] while still others have found no alteration in DHA binding in either the dependent or withdrawn state [37]. These discrepancies may be due, in part, to strain differences. Thus, it is possible that F-344 rats, which exhibited no significant alteration in either clonidine or DHA binding in the dependent state, may be subject to changes in binding during withdrawal. Studies directed at this issue are currently in progress.

In view of the many discrepancies in the literature, and the present demonstration of strain differences in adrenergic ligand binding following chronic morphine, it would seem that alterations in adrenergic binding sites are not prerequisite for the expression of tolerance and physical dependence. Another consideration, however, is the possibility that adrenergic receptor sensitivity as expressed through secondary messengers may change in the absence of observable alterations in radioligand binding.

The data show that SPD binding in the striatum is increased in F-344, and decreased in BUF rats after chronic morphine. While LSD-displaced SPD binding is considered selective for 5-HT_2 binding sites in the frontal cortex and hippocampus [3,13], a number of studies indicate that this ligand system also labels dopamine D_2 sites in the striatum [12, 38, 41]. It has been estimated that approximately 15–20% of the total specific LSD-displaced SPD binding in the striatum represents 5-HT_2 sites [55]. Previous studies have shown that the highest density of 5-HT_2 sites occurs in the frontal cortex [3,33]. The present data, however, shows twofold higher levels of SPD binding in the striatum, as compared with frontal cortex. It is likely, therefore, that our assay system was labelling primarily D_2 binding sites in the striatum.

The fact that striatal SPD binding was significantly higher (23%) in F-344, as opposed to BUF rats, further indicates that these are D_2 sites, since a previous study has shown a 20% greater density of D_2 sites in the striatum of F-344 as compared with BUF rats [19]. If the alterations in striatal SPD binding following chronic morphine do reflect changes in D_2 binding, the strain difference seen here may be relevant to studies on development of dopaminergic supersensitivity after chronic morphine treatment. While enhanced behavioral responses to dopamine agonists following chronic opiate treatment are well documented, parallel increases in striatal dopamine binding are not consistently present [8]. Furthermore, both increases [20,32] and decreases [25,26] in dopaminergic function have been observed after chronic morphine treatment. These discrepancies may also be due, in part, to the strains of rats employed for study. Once again, however, alterations in secondary messengers in the absence of observable changes in ligand binding are a possibility.

The literature concerning opiate relationships with serotonergic function is more consistent than that for norepinephrine [17, 21, 22, 23, 53]. The present study demonstrates that 5-HT_2 binding sites are increased in the hippocampus of both F-344 and BUF rats after chronic morphine. This alteration is particularly significant in light of recent studies demonstrating that "wet dog shakes" associated with morphine withdrawal are serotonergically mediated [2] through 5-HT_2 mechanisms [55]. Furthermore, the hippocampus has been shown to be an important brain region in terms of immunofluorescent localization of methadone distribution [42] and induction of withdrawal behaviors [24,54]. Finally, recent evidence indicates that the administration of drugs which enhance serotonergic function results in decreased morphine intake in dependent rats during choice tests [57]. Thus, it could be speculated that a role exists for serotonergic mechanisms in opiate action.

The results of these experiments are preliminary in the sense that saturation analyses will be required to determine the relative contributions of Bmax and Kd to observed binding alterations. Tissue volume limitations prevented assay of all three ligands at more than one concentration, however, each ligand was assessed at a concentration near the apparent Kd for the binding site being examined [7, 48, 52]. Experiments are in progress to further characterize the nature of binding sites which were found to be significantly altered by morphine treatment. Behavioral and biochemical studies are also in progress to determine the validity of a potential relationship between serotonergic function and morphine withdrawal syndrome.

In conclusion, we have shown that BUF and F-344 rats differ with respect to both baseline and morphine-induced alterations in radioligand binding. Despite these strain differences, a consistent increase in hippocampal 5-HT_2 binding was observed after chronic morphine consumption. These results emphasize the value of the strain difference model as it may be employed to clarify discrepancies in the literature. Of even greater significance is its power in identifying strain-independent neurochemical alterations in response to drugs such as morphine, which elicit strain-independent behaviors such as tolerance and physical dependence.

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